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Prof. Dr. A. E. Melchinger

Resistance of Maize (*Zea mays* L.) Against the European Corn Borer (*Ostrinia nubilalis* Hb.) and its Association with Mycotoxins Produced by *Fusarium* spp.

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Thomas Magg
aus
Bronnen (Kreis Biberach an der Riß)

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	3. Prüfer:	Prof. Dr. W. Claupein

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Abbreviations

15-A-DON	15-acetyl-deoxynivalenol
3-A-DON	3-acetyl-deoxynivalenol
ANOVA	analysis of variance
BC	backcross
Bt	*Bacillus thuringiensis*
cm	centimeter
DIMBOA	2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one
DON	Deoxynivalenol
DRS	damage rating of stalks
ECB	European corn borer
EPA	Environmental Protection Agency
FUM	Fumonisin
FUS-X	Fusarenon-X
g	gram
GC-ECD	gas chromatography with electron capture detection
GDI	grain dry matter of infested plots
GDP	grain dry matter of protected plots
GMO	genetically modified organisms
GR	genetic ratio
GYI	grain yield of infested plots
GYP	grain yield of protected plots
GYR	grain yield reduction
h^2	heritability
ha	hectare
HPLC	high pressure liquid chromatography
INRA	Institut National de la Recherche Agronomique
LPE	number of larvae per ear
LPP/LAV	number of larvae per plant
LSD	least significant difference
m	meter
M	mortality
MON	Moniliformin
NIV	Nivalenol
P	probability
PDE	percentage of damaged ears
PDP	percentage of plant displaying ECB feeding damage
PHI	height of infested plant
PHP	height of protected plants
QTL	quantitative trait loci
RCBD	randomized complete block design
RFLP	restriction fragment length polymorphism
$r_{p/g}$	phenotypic/genotypic correlation coefficient
S_x	selfing generation x
SD	standard deviation
SSR	simple sequence repeat
t	ton
TC	testcross
TCTC	Trichothecene
TL	tunnel length
ZEN	Zearaleon

GENERAL INTRODUCTION

The European corn borer *Ostrinia nubilalis* Hübner, (ECB) is one of the most important insect pests (*Lepidoptera: Pyralidae*) in maize (*Zea mays* L.) production in Central Europe. European corn borer larvae cause yield losses of up to 30% in regions with a high natural occurrence of ECB due to feeding and tunneling in plants (Jarvis et al. 1984, Bohn et al. 1998). Furthermore it is assumed that ECB damage favors secondary mold infections such as *Fusarium* spp. or *Ustilago maydis*, which may led to additional yield losses and adversely affect the quality of grains (Lew et al. 1991, Munkvold et al. 1997, 1999).

Biology of the European corn borer

The ECB originated in Europe and expanded to the Middle East, northern Africa, North America, and Central America (Hoffmann und Schmutterer 1999). The Z-race of ECB primarily depends on maize as the host plant and, therefore, developed into an important maize pest since maize production rapidly increased in Central Europe (Langenbruch and Szewczyk 1995). In contrast, the polyphagous E-race attacks a wide range of herbaceous wild and cultivated plant species with stems large enough for larvae to enter, *e.g.*, *Polygonum* spp., *Utrica* spp., and *Solanum tuberosum* L. (Hudon and LeRoux 1986).

In Central Europe the ECB completes normally one generation (univoltine) per year. In warmer regions, ECB occurs with two or more generations (multivoltine), depending on the geographic latitude and regional climatic conditions. Moths of ECB occur mid of June until the second half of July and oviposit egg masses onto maize plants in the late whorl stage before anthesis (Figure 1). After 10 to 14 days larvae begin to hatch. First- and second-instar larvae feed initially on leaf tissue within the whorls and then attack the enclosed tassels, feeding on the developing anthers. Later instars prefer pollen that accumulate in the leaf axils, attack the ears and shanks before boring into the stem of the plant. The adult larvae feed extensively in the stalks moving downwards to the bottom of the stalk or inside the ear to diapause (Hoffmann and Schmutterer 1999).

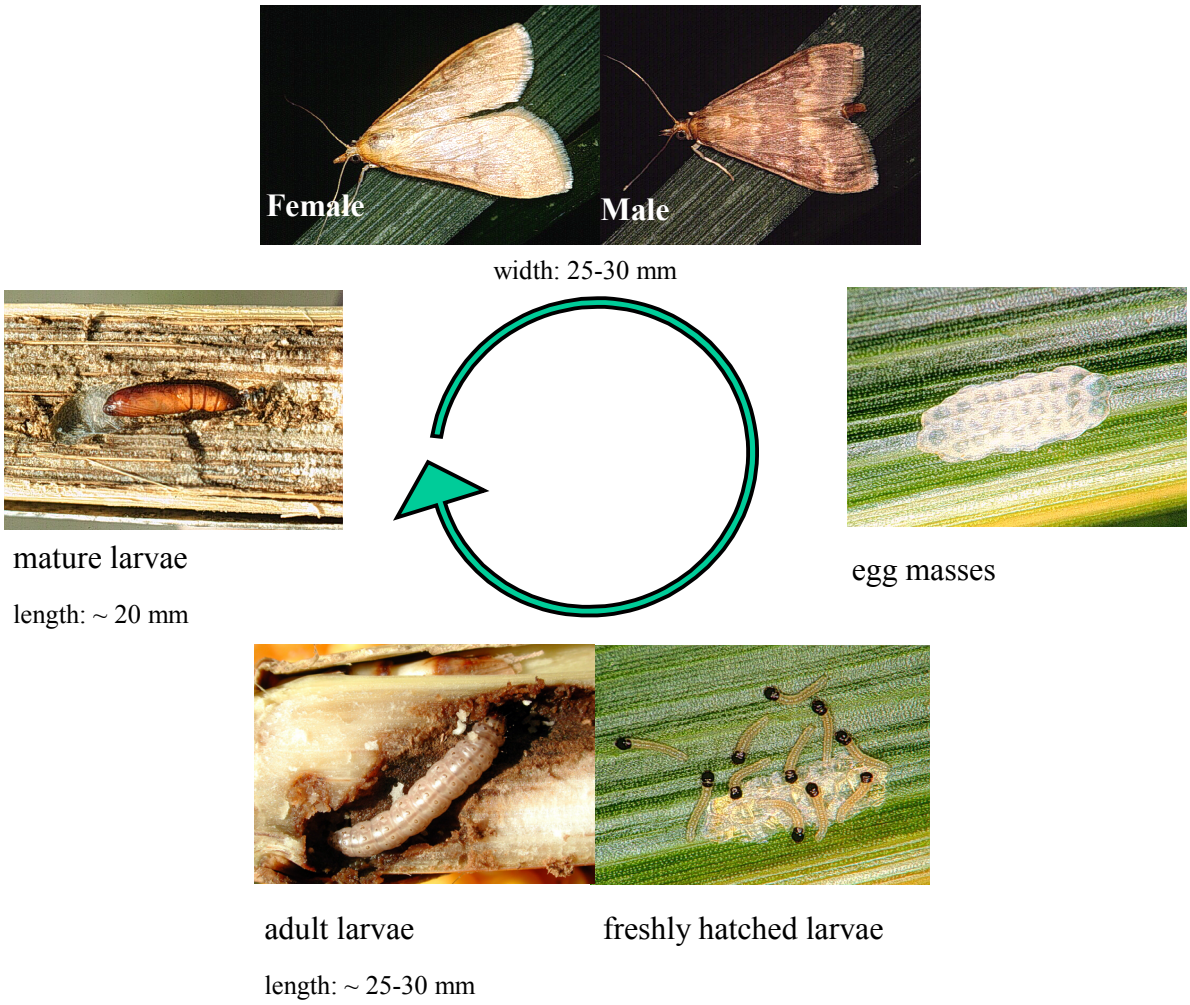

Figure 1: Life cycle of the ECB (*Ostrinia nubilalis* Hübner).

Methods for controlling European corn borer damage

In many maize growing areas, ECB populations exceed the economic threshold and, therefore, farmers are forced to take control measures (Rost 1996). The traditional ECB management method is to destroy shelter for overwintering by crushing maize residues and plowing. Furthermore, various insecticides (pyrethroid or organophosphate insecticides) as well as bacterial (*Bacillus thuringiensis, Bt*) and biological (*Trichogramma* parasites) control methods for ECB are available. However, ECB larvae on maize plants are difficult to combat, because they are exposed to sprays or antagonists for only a short period of time before they bore into the plant.

Improving natural host plant resistance is an economically and ecologically promising mean to control ECB infestation. The natural host plant resistance against ECB can be based on three resistance mechanisms: (i) nonpreference, (ii) antibiosis, and (iii) tolerance (Painter 1968, Panda and Khush 1995). Nonpreference is a lack of attractiveness for oviposition and could be evaluated in laboratory trials and field experiments (Guthrie and Barry 1989, Orr and Landis 1997). Antibiosis to the first ECB generation (leaf feeding resistance) is mainly based on the concentration of the chemical compound DIMBOA [2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one] in the leaves. In addition, antibiosis to the second ECB generation depends on a number of factors such as the concentration of detergent fiber, cellulose, lignin, and biogenic silica in cell walls and tissue toughness (Bergvinson et al. 1994, Ostrander and Coors 1997). Several studies investigating resistance to insects have focused on antibiosis by either evaluating larval growth or mortality in field or laboratory studies or by assessing stalk feeding damage of plants under manual infestation of ECB (Wiseman et al. 1981, Hudon and Chiang 1991, Jansens et al. 1997). In contrast, tolerance is the ability of a maize plant to withstand a certain population density of ECB without loss of yield or increased stalk breakage (Painter 1968, Barry and Darrah 1997).

Control of ECB damage through *Bt* hybrids

An alternative approach to control ECB damage is the use of transgenic maize hybrids, carrying genes isolated from the soil borne *Bt* var. *kurstaki* HD-1. The used δ-endotoxins are predominantly fatal to Lepidopteran insect species such as ECB or, depending on the used *Bt* strains, to beetles (*e.g.*, Western corn rootworm, *Diabrotica virgifera virgifera*) and aquatic flies (*e.g.,* Mosquitoes, *Anopheles stehpensi*) (http://www.colostate.edu/programs/lifesciences/TransgenicCrops/BtQnA.pdf, confirmed 02.05.2002). After *Bt* uptake by the insect, the crystalline protein dissolves to release a toxin that attacks the gut lining (Meeusen and Warren 1989). Feeding stops within a few hours. The insect gut wall breaks down within 24 hours. The insect dies from toxins attacking the gut wall by a general body infection (septicemia), which is present within 48 hours, and food deprivation (Sagers et al. 1997). The activity of the toxin in an insect depends on the gut's pH, the presence of enzymes and reducing agents, and the presence of protein binding sites on cell membranes.

The *Bt* gene *CryIA(b)* was one of the first genes that attracted the interest for use in plant transformation with lethal effects against Lepidopteran species (Meeusen and Warren 1989, Jansens et al. 1997, Archer et al. 2000). In plants, gene promoters regulate the tissue- and developmental stage-specific expression of the *Bt* gene. Based on the European Council Directive 90/220 and the German Seed Act, only maize hybrids derived from transformation events Mon810 and event 176, both containing the *CryIA(b)* gene, have so far a restricted license to be used in maize production in Germany. Mon810 utilizes a gene promoter, which results in a season-long expression of the *Bt* toxin in all plant tissues (Archer et al. 2000). In contrast, event 176 contains two promoters, one regulating *Bt* gene expression exclusively in green plant tissues and the other in the pollen (Koziel et al. 1993, Estruch et al. 1997).

The high level of resistance of *Bt*-transformed maize against ECB was demonstrated in several studies under U.S. growing conditions (Koziel et al. 1993, Estruch et al. 1997, Jansens et al. 1997, Pilcher et al. 1997, Sagers et al. 1997 and Archer et al. 2000). With the use of *Bt* hybrids the mortality of ECB larvae exceeds 99% (Gould 1994). Based on economic considerations, Bohn et al. (1998) concluded that *Bt* maize should be the most economic ECB control measure under Central European conditions because of its high level of resistance. However, no studies were available on the effectiveness of the *Bt* resistance against ECB in early maturing European maize germplasm.

The monogenic *Bt* resistance of maize hybrids may entail the risk of being overcome by resistant insect individuals, which occur in low frequencies in natural populations (Tabashnik 1994, Metz et al. 1995, Onstad and Gould 1998), or of being harmful to unrelated species such as the monarch (*Danaus plexippus*) (Losey 1999). Therefore, improving the non-transgenic host plant resistance of maize, governed by multiple genes, should be favored to ensure a sustainable integrated pest management. In order to increase the durability of the *Bt* gene, both types, the monogenic *Bt* resistance and the natural host pant resistance, could be combined.

Improving host plant resistance of maize against ECB

The host plant resistance of maize against the second ECB generation is quantitatively inherited and associated with at least seven genomic regions (Jennings et al. 1974). Bohn et al. (2001) found six quantitative trait loci (QTL) for tunnel length and five QTL for stalk damage rating, explaining about 50% of the genotypic variance in the early maturing European maize germplasm. Furthermore, diallel studies and generation mean analyses confirmed a mainly additive gene action and to a lesser extend dominance and epistatic interactions (Jennings et al. 1974).

In contrast to the U.S. Corn Belt maize germplasm, only few studies on the resistance of maize against ECB under Central European growing conditions are available. Therefore, a large number of elite inbreds of both opposite pools were screened for ECB resistance under manual infestation of ECB and a significant genetic variation was found for improving ECB resistance traits (Schulz et al. 1997, Kreps et al. 1998, Melchinger et al. 1998). However, breeding for resistance against the second generation of ECB has proven difficult. While backcross breeding appeared to have little effect in improving the level of resistance owing to the putatively high number of genes involved, recurrent selection resulted in a negative correlated selection response for other agronomically important traits such as yield (Guthrie and Russell 1989). After selection for ECB resistance, Klenke et al. (1986) observed reduced grain yield in maize synthetic BS9. Improved ECB resistance was also found to be associated with late flowering and late grain maturity in maize inbreds (Hudon and Chiang 1991, Schulz et al. 1997). Therefore, a breeding program was initiated at the University of Hohenheim in the mid 1990's to improve ECB resistance in early maturing germplasm and, at the same time, to avoid indirect selection of genotypes with late maturity and lower grain yield.

Infection of maize with Fusarium species

Species of *Fusarium* are among the most common fungal associates of maize plants causing diseases of seedlings, roots, stalks and kernels. In Central Europe the most common *Fusarium* species are *F. graminearum*, *F. culmorum*, *F. subglutinans, F. avenaceum*, and *F. moniliforme* (Lew 1993, Bottalico 1998). It is supposed that on average about 7% of the world

maize harvest is destroyed by stalk rots (Hoffmann and Schmutterer 1999). Fusarium molds are also capable of producing secondary metabolites that cause severe physiological and pharmacological responses in humans, animals, and plants (IARC 1993). Therefore, many countries initiated programs to set up mycotoxin thresholds in food and feed for human and animal consumption, respectively.

The contamination with mycotoxins produced by *Fusarium* spp. can especially be observed in regions, where single crop rotation systems are prevalent. Warm and humid weather conditions and the cultivation of late-ripening varieties favor the development of Fusarium species (Lew et al. 1991). Furthermore, mycotoxin production of *Fusarium* spp in infested maize plants is difficult to control. *In vivo* studies reported inconsistent results, from an inhibition to an accumulation of mycotoxin synthesis after fungicidal treatments (Hasan 1993). Since the mycotoxin concentration is stable under normal storage conditions and a detoxification with physical treatments like high temperatures, UV radiation, and oxygen are not effective (Patey and Gilbert 1989, Eriksen and Alexander 1998), resistance breeding or biotechnological approaches are economical and ecological means to improve host plant resistance against *Fusarium* spp.. However, only little is known about resistance mechanisms of maize against *Fusarium* spp. and their interaction in the host plant.

Fungi of the genus *Fusarium* spp. infect the maize ear through the silk channel or by using other pathways e.g., wounds caused by insects or birds, to incorporate into their host plant (Reid 1999, Reid et al. 1999). It is hypothesized that ECB larvae are vectors for *Fusarium* spp. by causing entry wounds and carrying fungi inoculum from the plant surfaces into the plant itself. Furthermore, a close association between susceptibility of maize hybrids to second generation ECB damage and the appearance of stalk rot exists (Jarvis et al. 1984). Studies showed that the use of *Bt* maize hybrids decreased FUM contamination of grains under U.S. Corn Belt environmental conditions (Munkvold et al. 1997, 1999). Austrian studies found under ECB larvae feeding an increase of the MON producing *Fusarium* species, whereas the frequency of other *Fusarium* strains was considerably reduced (Lew et al. 1991, Lew 1993). Yet no prior information exists on the potential of highly ECB resistant *Bt* hybrids reducing the level of mycotoxin contamination in maize under Central European growing conditions.

The overall goals of this study were to develop maize germplasm with improved non-transgenic host plant resistance against ECB by employing conventional breeding methods and to explore the efficacy of *Bt* maize hybrids grown under Central European growing conditions.

The objectives of the present study were to:

(1) initiate a selection experiment in the early maturing European flint pool and evaluate a breeding program for ECB resistance in the European dent pool,

(2) compare the efficiency of host plant resistance *vs. Bt* resistance in maize,

(3) determine *Fusarium*-caused mycotoxin contamination of maize genotypes with improved host plant resistance to ECB, and

(4) study the association between important agronomic traits, ECB resistance traits, and mycotoxin concentration in early European maize germplasm.

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Figure 1: T. Magg (University of Hohenheim), http://www.ent.iastate.edu/imagegal/lepidoptera, http://www.uwrf.edu/~cg04 /333/ecb.

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BREEDING EARLY MATURING EUROPEAN DENT MAIZE (*ZEA MAYS* L.) FOR IMPROVED AGRONOMIC PERFORMANCE AND RESISTANCE AGAINST THE EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* HB.)

M. Bohn[1*], T. Magg[2], D. Klein[2], A.E. Melchinger[2]

[2] *Institute of Plant Breeding, Seed Sciences, and Population Genetics, Univ. of Hohenheim, 70593 Stuttgart, Germany*

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ABSTRACT - The prime objective of maize breeding programs in Western Europe is improving yield, maturity, and stalk quality. Increasing the resistance to the European corn borer (*Ostrinia nubilalis* Hb., ECB) is of secondary importance, despite its significance as a major pest of maize in Western Europe. Given this priority setting, the overall goal of the Hohenheim ECB breeding program, initiated in 1992, was to select lines with improved *per se* and testcross performance for multiple agronomic traits and ECB resistance. Objectives of this study were to describe the used breeding scheme and the results obtained after ten years of intensive selection work. In the standard breeding scheme, line development started from a segregating S_1 population. Genotypes were evaluated for their line *per se* ECB resistance in generations S_1, S_3, and S_5. Lines from the S_2, S_4, and S_5 generations were testcrossed and evaluated for their agronomic performance. Selection was based on ECB resistance and TC performance for grain yield and maturity. The five newly developed lines showed only a minor improvement for ECB resistance. This can be explained by the negative correlation between the used selection index for agronomic traits and ECB resistance. The population fraction selected based on index performance did not contain the lines with the highest level of resistance. None of the selected lines showed associations between SSR haplotypes at ECB resistance gene clusters and their level of ECB resistance. These results demonstrated that a simultaneous improvement of important agronomic traits and ECB resistance is difficult to accomplish, if conventional tools typically used by maize breeders are applied. Further research is needed to develop new breeding methods to overcome these limitations.

KEY WORDS: Line development; Respose to selection; Selection index; Testcross performance.

INTRODUCTION

The European corn borer (ECB), *Ostrinia nubilalis* Hb., is a major pest of maize (*Zea mays* L.) in Germany. From its original area of occurrence in the Rhine Valley and in Bavaria, the ECB has now spread to the more northerly maize growing regions of the Köln-Bonner Bucht and the Oderbruch (LANGENBRUCH and SZEWCZYK, 1995). Damage is caused by ECB larvae feeding in stalks and ears reducing both quantity and quality of the grains. Yield losses in the magnitude of 30dt ha^{-1} and more have been observed in Baden-Württemberg (BOHN *et al.*, 1998; MAGG *et al.*, 2001) and in the U.S. Cornbelt (JARVIS *et al.*, 1984). The entry of secondary fungal pathogens such as stalk- or ear rot (*Fusarium* spp.) and cornsmut (*Ustilago maydis*) is facilitated due to the mechanical tissue damage caused by the larvae (JARVIS *et al.*, 1984; LEW *et al.*, 1991; MUNKVOLD *et al.*, 1999; MAGG *et al.*, 2002). Furthermore, *Fusarium* spp. diseases may produce mycotoxins with detrimental effects to humans and animals (IARC, 1993).

The ECB can be managed mechanically through mulching and plowing under of maize stalks, chemically through pyrethroids or organophosphates, or biologically using parasitic *Trichogramma* wasps (*Trichogramma evanescens*) (HOFFMANN and SCHMUTTERER, 1999). Furthermore, conventional maize breeding can reduce yield losses by improving natural host plant resistance against ECB. Transgenic *Bt* hybrids carrying the transformation events "event 176" and "Mon810" are licensed to restricted use in Germany since 1998 (EU-directive 90/220/EWG). However, the general public is opposed to the use of transgenic plants. Therefore, improved natural resistance against ECB can represent an economically and ecologically viable alternative to *Bt* resistance for farming.

In contrast to the North American Cornbelt, where ECB may have several generations, it only

[1] Present address: S-110 Turner Hall, Crop Sciences, University of Illinois, 1102 South Goodwin Avenue, Urbana, IL 61801, USA.

* For correspondence (email: mbohn@uiuc.edu).

has one generation per year in Germany. The time of natural infestation and damage caused by the first ECB-generation in Central Europe correspond to that of the second ECB-generation in North America. Genetic studies have demonstrated that resistance against the second generation of larvae is inherited quantitatively (SCHÖN *et al.*, 1993; RUSSELL *et al.*, 1974). The resistance is probably based on cell wall fortification promoted by additional inclusions of cellulose, lignin, and biogenic silicates (BERGVINSON *et al.*, 1994).

Efficient methods to assess ECB resistance of maize genotypes and detailed information on the genetic variation for the used resistance traits are key prerequisites for improving ECB resistance employing conventional breeding strategies. SCHULZ *et al.* (1997) and MELCHINGER *et al.* (1998) identified significant genetic variation for ECB resistance traits damage rating of stalks (DRS) and relative tunnel length (TL) evaluating 115 European Flint and Dent lines. Using European maize breeding material, KREPS *et al.* (1998) showed a close, positive relationship between line *per se* performance and topcross performance with respect to ECB resistance. Furthermore, diallel studies in U.S. maize germplasm showed that general combining ability (GCA) is of greater importance for first and second generation ECB resistance than specific combining ability (SCA) (JENNINGS *et al.*, 1974; KIM *et al.*, 1989; THOME *et al.*, 1992).

At the University of Hohenheim, a breeding program was initiated in 1992 to improve grain yield and maturity along with the natural host plant resistance of maize against ECB larvae feeding in early maturing European dent germplasm. The objectives of this study were to describe the used breeding scheme and the results obtained after ten years of intensive selection work, and to discuss the lessons we have learned for designing breeding programs to improve both agronomic characteristics and ECB resistance.

MATERIAL AND METHODS

Breeding scheme

The overall goal of the Hohenheim ECB breeding program was to select at least one maize line per biparental source population with improved *per se* and testcross performance for grain yield, maturity, and ECB resistance. The breeding scheme was designed on the basis of the following information: (1) the association between line *per se* and testcross performance for ECB resistance was high in early maturing European dent germplasm and (2) the genotypic variance for ECB resistance traits DSR and TL was larger among lines than among their testcrosses (Kreps *et al.*, 1998). In the standard breeding scheme, line development started from a non-inbred S_0 population derived from the cross of two maize lines (Fig. 1). Individuals from this population were selfed to produce a segregating S_1 source population. Genotypes were evaluated for their line *per se* ECB resistance in generations S_1, S_3, S_5, and S_7. Lines from the S_2, S_4, and S_5 generations were testcrossed and evaluated for their agronomic performance. Lines in the S_3, S_5, and S_7 generations were selected based on their level of ECB resistance and the TC performance of their parental lines.

Germplasm screening

In order to identify promising maize lines as parents for starting a conventional breeding program, a total of 63 early maturing (< FAO 300) European dent inbred lines were tested for resistance against ECB in two environments in 1993 (MELCHINGER *et al.*, 1998). Based on this preliminary evaluation a selected sample of 23 dent lines was repeatedly tested for ECB resistance in two environments in 1994 (SCHULZ *et al.*, 1997). Further background information on the screened inbred lines is given by the companion publications SCHULZ *et al.* (1997) and MELCHINGER *et al.* (1998).

Selection of parental lines and procuring initial variation

Based on the germplasm screen, 19 dent lines with good ECB resistance and good agronomic performance were selected and crossed in a factorial manner to obtain 45 single cross hybrids. Selected single cross hybrids were crossed to produce 120 double cross hybrids. Out of this set of hybrids three single cross hybrids and one double cross hybrid, which was developed out

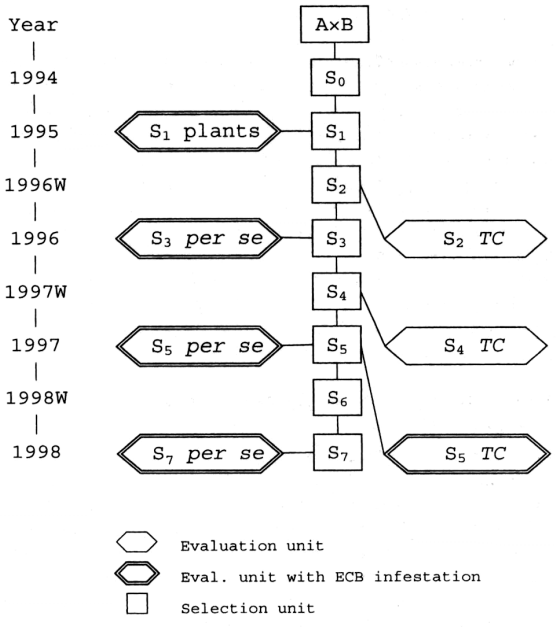

Figure 1 - Flow chart of the breeding scheme used to improve grain yield, maturity, and ECB resistance of early maturing European Dent maize lines at the University of Hohenheim.

TABLE 1 - *Mean* per se *performance of European Dent inbred lines for relative grain yield (RGY), damage rating of stalks (DRS) and tunnel length (TL) below the ear.*

Line	Origin	RGY†	DRS	TL
		%	1-9	cm
D06	University of Hohenheim	105a‡	2.7a	12.3a
D61	University of Hohenheim	83b	3.7b	11.7a
D67	University of Hohenheim	75b	3.6b	8.3a
RZ05	FAP Zürich	78b	2.6a	8.7a
KW5361	KWS SAAT AG	80b	2.7a	8.4a
Mean§		76	3.2	12.1
Minimum-Maximum		53-105	2.3-5.1	7.0-24.1
LSD 5%		18	0.7	6.3

† Yield under infestation with ECB relative to yield obtained under insecticide protection.

‡ Means with different letters are significantly different at probability level $P < 0.05$.

§ Mean, minimum, maximum, and LSD values were obtained from a set of 23 European Dent lines used in the study by SCHULZ *et al.* (1997).

of two selected single cross hybrids, were used for source population development (Table 2). In addition, single cross hybrid [RZ05xD61] was backcrossed to each parent. Single and double cross hybrids were selfed to produce segregating S_1 populations. The six source populations A1, A2, A3, B, C, and BC of the Hohenheim ECB breeding program were based on lines D06, D61, D67, RZ05, and KW5361 (Table 1).

Selection of inbred lines for hybrid development

Starting with segregating S_1 and BC_1 populations a standard pedigree breeding program was used for inbred line development. Individual plants per row were selected and selfed and grown ear to row in the following growing cycle (FEHR, 1991). Line development continued to the S_6 generation using two growing cycles per year (summer nursery in Eckartsweier, Germany; winter nursery in Bethlehem, South Africa). Lines from the S_2, S_3, and S_4 generation were testcrossed to testers from the Flint pool. The Flint single cross hybrid D149xD171 was used as tester for the S_2 lines and the Flint lines D171 and F012 were used as testers for the S_3 and S_4 lines. The testcross progenies (TC) were produced in the winter nursery.

Evaluation of inbred lines for ECB resistance and agronomic traits

Infestation with ECB larvae

Plants were manually infested with freshly hatched ECB larvae in order to assure an even infestation level. Timing of manual infestations and insecticide treatments were synchronized with light-trap catches of ECB adults. In general, the first ECB moths were consistently observed in mid June (June 15-20) and the last adults were caught in the second half of July (July 21-26). All plants in the insecticide protected rows were individually treated three times from the end of June to the beginning of August with an insecticide (FASTAC SC©) at 10 to 14 day intervals to prevent natural infestation with ECB. For manual infestation of lines and their TC progenies, ten plants in the center of a row were infested. An average of 20 ECB larvae was applied three times at weekly intervals for a total of about 60 larvae per plant.

Most maize plants were in the mid-whorl stage at the time of first infestation and tasseled or started silking at the time of the third infestation. Egg masses for rearing of ECB larvae were supplied by the Institut National de la Recherche Agronomique, Le Magneraud, France. After incubation, neonate larvae were mixed with corncob grits and placed into the whorl and leaf collar of the maize plants using a mechanical dispenser.

For the line *per se* evaluation, genotypes were planted in single row plots with 15 plants per row and two replications for evaluating agronomic characters during line development. Resistance against ECB was evaluated by manually infesting the six center plants of each row in the summer nurseries. The testcross progenies of S_2 (S_2 TC), S_3 (S_3 TC), and S_4 (S_3 TC) lines were evaluated in field trials together with testcrosses of their respective parents and commercial hybrids recommended for planting in the state of Baden-Württemberg, Germany. The TC performance of 141 S_2 lines was determined at two locations in 1996, whereas the TC performance of 26 S_3 lines was determined at three locations in 1997. The testcross progenies of 38 S_4 lines were evaluated at two locations in 1998. All experimental sites were located in the Upper Rhine Valley in southwest Germany. The S_2-TCs were split into two sets to accommodate the large number of testcrosses. The two sets were evaluated in adjacent field trials. In all experiments with manual ECB larvae infestation (*per se* evaluation of S_1 lines and *per se* and TC evaluation of S_4 lines), the experimental design applied at each site was a split plot with two replications. Whole plots consisted of two ECB treatments, one with ECB larvae infestation and the other with insecticide protection. Subplots were arranged according to an α-design. The subplots comprised the genotypes and consisted of single- or two-row plots, each row 4 m long with 25 plants and a row spacing of 0.75 m. Plants were machine-sown and manually thinned to a density of 83,333 plants per ha. In all experiments without ECB, larvae genotypes (TC evaluations of S_2 and S_3 lines) were evaluated in an α-design with two replications at each location.

Agronomic traits

Agronomic traits recorded for each plot were: (1) grain yield (GY), grain yield under insecticide protection (GYP), or grain yield under ECB infestation (GYI), in t ha^{-1} adjusted to 15.5% grain moisture. (2) Grain yield reduction (GYR) was calculated for each genotype in % by applying the formula GYR=(1-GYI/GYP)x100. (3) Grain dry matter content (GD) in % was also determined under insecticide protection (GDP) and under ECB infestation (GDI).

The following selection index was employed to identify improved lines in years 1996 and 1997:

$$\text{Index} = \left(2 \times \frac{GDP_i \times 100}{\overline{GDP}} + \frac{GYP_i \times 100}{\overline{GYP}}\right) - 300$$

Here, $\overline{GDP}_i$ and $\overline{GYP}_i$ denote the grain dry matter and the grain yield of the i^{th} individual under insecticide protection, respectively. The variables GDP and GYP are the mean grain dry matter and the mean grain yield of the commercial hybrids used as checks, respectively. In addition, lines in all generations and their TC progenies were screened for field appearance, fertility, early vigor, root and stalk lodging as well as for infections caused by Fusarium and corn-smut.

TABLE 2 - *Mean* per se *performance of the tested and selected fraction of S_1 populations for ECB resistance traits damage rating of stalks (DRS) and tunnel length (TL) evaluated at three locations in 1995.*

Population	Pedigree	Lines		Selected Fraction			
		Total	Selected	DRS	S_{DRS}+	TL	S_{TL}
		No.		1-9		cm	
A1	(RZ05 x D61)	113	30	2.2±1.2‡	–0.6	0.6±0.8	–6.1
A2	(RZ05 x D61)xD61	45	25	3.2±2.1	–0.1	4.7±3.1	–5.1
A3	(RZ05 x D61)xRZ05	78	25	1.6±1.0	–0.8	0.9±0.9	–6.0
B	(RZ05 x D67)	130	30	2.0±1.0	–0.5	0.1±0.4	–5.4
C	(KW5361 x D06)	76	30	2.6±1.6	0.1	0.4±0.7	–6.2
BC	(RZ05 x D67)x(KW5361 x D06)	26	12	2.7±1.2	0.2	1.1±1.0	–3.9
Mean		468	152	2.3±1.4	–0.3	1.2±2.1	5.5

‡ Standard deviations are attached to the mean of the tested fraction.
+ S = selection differential.

ECB resistance traits

Resistance to ECB larval feeding was measured for each plant in the infested plots by (1) longitudinally splitting the stalks to measure the TL caused by larvae feeding in cm and (2) assessing the feeding damage using a 1 to 9 damage rating scale (DRS) (1 for no visible symptoms, 2-3 for broken tassels, 4-8 for stalk breakage above the ear, and 9 for plants broken below the ear), as described by HUDON and CHIANG (1991).

Statistical Analysis

Data of individual plants was averaged to obtain plot means. The analysis of variance for the S_2 and S_3 line *per se* and TC experiments were performed according to a randomized complete block design (RCBD). The TC progenies of the S_4 lines were analyzed following the procedures for a split-plot experimental design. Combined analyses across locations were performed using adjusted means. Least significant differences (LSD 5%) and stan-

TABLE 3 - *Mean and selection differential* (S) *of populations of S_2 lines testcrossed to D149xD171 for grain yield (GYP), grain dry matter content (GDP) under insecticide protection, as well as for line* per se *performance for damage rating of stalks (DRS) evaluated at two locations in 1996.*

Characterization	Lines		GYP		GDP		Index†		DRS‡	
	Total	Selected	$\bar{\chi}$	S_{GY}	$\bar{\chi}$	S_{GD}	Mean	S_{Index}	$\bar{\chi}$	S_{DRS}
	No.		dt ha^{-1}		– % –				– 1-9 –	
Trial 1§										
A1	12	1	85.4	21.2	63.9	–0.5	–18.2	13.5	2.7	0.2
A2	36	6	95.4	4.3	63.8	–0.2	–8.5	4.7	2.7	0.5
A3	25	0	88.1	–/–	64.4	–/–	–13.9	–/–	2.4	–/–
Mean			91.9		64.1		–11.1		2.6	0.4
LSD 5%			18.0		0.8		19.0			
Trial 2										
B	35	1	91.1	11.9	64.1	0.3	–10.9	10.6	2.7	–1.3
C	21	5	92.0	2.2	65.6	0.6	–5.4	3.7	2.7	–0.1
BC	12	4	96.3	–3.0	65.2	1.5	–2.6	1.5	2.5	–0.5
Total	141	17								
Mean			92.8		64.8		–7.2		2.6	–1.0
LSD 5%			13.1		0.9		14.6			

† Index formula is given in the Materials and Methods.
‡ Damage rating of stalks (DRS) were obtained from a *per se* evaluation of lines in the summer nursery.
§ Due to the large number of entries (N=141), the experiment was divided into two trials (Trial 1 and Trial 2). Both trials were grown in adjacent field plots.

TABLE 4 - *Testcross performance of S_4 lines selected for grain yield (GY) and grain dry matter content (GD) using a selection index evaluated at three locations in 1997 with two flint testers F012 and D171.*

Population	Entries	GYP		GD		Index†	
		F012	D171	F012	D171	F012	D171
	— # —	dt ha^{-1}		%			
A1	1	83.1	72.8	69.0	70.8	–9.0	–7.4
A2	6	84.3	76.8	69.4	70.3	–7.1	–2.5
B	1	84.1	72.0	69.3	70.3	–7.6	–9.1
C	5	84.9	72.6	72.4	73.0	1.1	–0.6
BC	6	75.5	68.7	72.4	73.7	–8.4	–7.5
Total mean		84.5	74.4	71.0	71.7	–3.2	–2.1
LSD 5%		11.0	10.6	1.3	1.3	12.7	13.5

† The Index is given in the Material and Methods.

dard deviations (SD) were determined as described by SNEDECOR and COCHRAN (1980). All required calculations were carried out with software package PLABSTAT (UTZ, 1998).

RESULTS

Selection of parental lines and procuring initial variation

Significant genetic variation ($P < 0.01$) for agronomic traits as well as for ECB-resistance was found in a set of 63 early maturing European elite maize Dent lines (see Tables 2 in MELCHINGER *et al.*,1998, and SCHULZ *et al.*, 1997). The five lines selected as parents for our breeding program showed no significant differences for TL below the ear (Table 1). Damage ratings of stalks were significantly ($P < 0.05$) smaller in lines D06, RZ05, and KW5361 (DRS ≤ 2.7) than in lines D61 and D67 (DRS ≥ 3.6). Relative grain yield varied between 75% (D67) and 105% (D06). Among the six S_1 populations the mean DRS values ranged from 2.4 (A3) to 3.3 (A2) with a mean value of 2.6 (Table 2). The mean TL below the ear varied between

TABLE 5 - *Means of S_5 TC for grain yield under insecticide protected conditions (GYP), grain yield reduction (GYR), and grain dry matter content (GDP) as well as means for the damage rating of stalks (DRS) of selected S_7 lines and their parents. The trial was carried out in two locations in 1998.*

Pedigree	Population	GYP		GYR		GDP		DRS	
		F012	D171	F012	D171	F012	D171	F012	D171
		dt ha^{-1}		%				1-9	
Parent									
RZ05		83.0	67.3	11.2	12.5	68.6	67.4	4.2	3.6
D67		79.5	81.6	20.1	21.1	70.5	68.4	5.2	5.6
D06		70.3	70.6	24.1	25.2	72.9	71.6	6.4	5.3
KW5361		80.6	83.2	28.0	27.4	70.0	68.7	5.7	5.0
D61		82.7	82.1	32.7	34.1	69.3	67.4	5.3	5.1
Mean		79.2	77.0	23.2	24.1	70.3	68.7	5.4	4.9
S_4 lines									
P028	B	83.5	74.8	24.0	17.3	69.2	67.5	5.2	4.5
P029	C	73.9	78.5	25.6	19.3	71.8	69.8	5.5	5.0
P030	C	77.5	77.2	27.1	22.5	72.6	71.1	5.0	4.7
P031	BC	79.4	73.3	26.3	27.6	71.4	69.6	5.5	4.8
P032	A2	74.6	75.4	27.7	18.7	69.2	68.3	4.9	4.8
Mean		77.8	75.8	26.1	21.1	70.8	69.3	5.2	4.8
Total mean		78.5	76.4	24.7	22.6	70.5	69.0	5.3	4.8
LSD 5%		11.1		8.6		0.8		1.0	

5.0 cm (BC) and 9.8 cm (A2) among populations with a mean of 6.6 cm. Per population, between 23% (B) and 56% (A2) of the tested S_1 plants were selected, *i.e.*, selection intensities of 1.32 and 0.80, respectively, resulting in an average selection differential of –0.3 for DRS and –5.5 cm for TL below the ear.

Selection of inbred lines for hybrid development

Highly significant ($P < 0.01$) differences were found for S_2 TCs within and between source populations for all traits (Table 3). The population means for GYP varied between 85.4 dt ha^{-1} (A1) and 96.3 dt ha^{-1} (BC) across trials with an average GYP of 91.9 dt ha^{-1} in Trial 1 and 92.8 dt ha^{-1} in Trial 2. The GDP ranged from 63.8% (A2) to 65.6% (C) with an overall mean of 64.5% in both trials. The populations displayed low to high repeatabilities for GYP ($0.14 < R < 0.86$) and moderate to high repeatabilities ($0.68 < R < 0.86$) for GDP. The selection index combining GYP and GDP ranged from –18.2 (BC) to –2.6 (A1). The mean TC performance of parents and S_2 TC progenies were not significantly different (data not shown).

Mean values of DRS varied between 1.9 (RZ05) and 3.6 (D67) for the parental lines (data not shown) and between 2.4 (A3) and 2.7 (A2, B, C) for the populations (Table 3). Seventeen out of 141 S_2 lines were selected based on their TC performance for GY and GD and their line *per se* performance for ECB resistance. Across populations the selection differentials varied between 1.5 and 13.5 for the selection index (Table 3). The means of the selected lines were not significantly different from the respective population means for GDP and DRS.

Grain yield means under insecticide protection of the 19 selected S_4 TCs varied from 65.7 dt ha^{-1} (BC) to 84.9 dt ha^{-1} (C) and the mean GD varied from 69.4% (cross) to 73.7% (BC) (Table 4). Significant ($P < 0.05$) differences among the selected S_4 lines were only found for testcrosses with tester line F012. Population A1 was excluded from the further breeding program because of severe fertility problems. The 18 selected S_4 lines were advanced to 38 S_5 lines.

Across all S_5 TCs, GYP ranged from 67.3 to 83.5 dt ha^{-1} and GYR varied between 11.2 and 34.1% (Table 5). GDP ranged from 67.4 to 72.6% and DRS varied between 3.6 and 6.4. Based on their testcross performance, five S_5 lines were selected. Significant differences were not found between the TC performance of parents and their selected S_5 lines for all agronomic and resistance traits. Differences between testers F012 and D171 were not significant.

DISCUSSION

The main objectives of conventional maize breeding programs in Western Europe are to increase grain and silage yield as well as to improve stalk quality and maturity, the latter to adapt maize to cooler Northern European growing conditions. All other traits, like resistances to fungi, viruses, or insect pests and quality characteristics, like high oil or specific starch configurations, are of secondary importance. Given this priority setting, we set up a breeding program to further agronomically enhance early maturing European Dent germplasm, while resistance to ECB larvae feeding was improved.

The introduction of monogenic resistances into elite breeding germplasm can be accomplished by continuous backcrossing using highly elite inbred lines as recurrent parents. In contrast, it is a much more complex endeavor to improve the quantitatively inherited host plant resistance against ECB. In order to improve ECB resistance in concert with grain yield and early maturity within the framework of a pedigree breeding program the following criteria must be meet: (1) The breeding material should possess a high genetic variation for ECB resistance. (2) The ECB resistance traits should allow an integration into existing breeding programs with little additional work and financial input. (3) For the simultaneous improvement of three traits (grain yield, early maturity, and ECB resistance) it would be ideal, if either the target traits were not correlated with each other or the correlation coefficients point in the direction desired by the breeder.

SCHULZ *et al.* (1997) and MELCHINGER *et al.* (1998) reported significant genotypic variance for relative grain yield, damage ratings of stalks, and tunnel length in early-maturing European maize germplasm. Based on these results, they concluded that ample genetic variation is available for improving ECB resistance in early maturing Flint and Dent germplasm. In addition, they identified among 115 maize lines, genotypes combining minimal yield reduction, low damage ratings of stalks, and short feeding cavities in stem. As MELCHINGER *et al.* (1998) proposed, these resistant inbreds with good agronomic performance were used as parental materials to develop the breeding populations of the Hohenheim program to improve ECB resistance.

We evaluated the overall level of ECB resistance of each genotype by determining grain yield differences between manually infested and insecticide protected plots. The grain yield differences are a

function of antibiosis, tolerance, and non-preference. In order to separate the effects of antibiosis from the effects attributable to the other resistance components, damage ratings of stalks and tunnel lengths were determined. The Hohenheim breeding program focused on the damage rating of stalks as the primary resistance trait for following reasons. The damage ratings were highly significantly correlated with grain yield reduction and tunnel length but showed higher heritabilities than these traits (KREPS *et al.*, 1998). The high heritabilities were mainly due to non-significant estimates of genotype x environment interaction variances. This allowed the reduction of the number of test locations without loosing validity. In addition, damage ratings displayed a closer correlation between line *per se* and TC performance than all other resistance traits (KREPS *et al.*, 1998). Beyond these reasons, damage rating of stalks could be evaluated with a fraction of the labor input and costs necessary to measure tunnel length or to determine grain yield reduction.

Grain yield and early maturity as well as grain yield and damage rating of stalks are negatively correlated. Highly significant and positive genotypic correlations were also reported between damage rating of stalks and early maturity (SCHULZ *et al.*, 1997; KREPS *et al.*, 1998; MELCHINGER *et al.*, 1998; MAGG *et al.*, 2001). In order to avoid unwanted correlated selection responses between agronomic traits and ECB resistance a two step selection procedure was applied. In the first step, genotypes with high grain yield and early maturity were selected employing a selection index combining both traits. In the second step, genotypes displaying a high level of ECB resistance were chosen from the previously selected fraction of the population. Alternative selection procedures to prevent negatively correlated selection responses might be the subdivision of materials according to their maturity into subgroups and testing each group in separate experiments, the correction of the resistance scores of genotypes for their maturity, similar to the calculation of maturity-corrected yield (UTZ *et al.*, 1978), or the extension of the selection index to account for yield, maturity, and ECB resistance.

However, the retrospect evaluation of the Hohenheim breeding program revealed only a minor improvement towards increased levels of ECB resistance in European early maturing Dent germplasm. This result can be explained by the independent culling levels employed for the used selection index and the ECB resistance. The success of this selection type is influenced by the phenotypic correlation between the traits used for selection. Due to the negative correlation between the selection index, which combined grain yield and maturity, and ECB resistance, the population fraction x selected based on its index performance did not contain the lines with the highest ECB resistance. In order to increase the probability to identify new lines that combine high index values with improved ECB resistance, repulsion phase linkage between genes involved in the inheritance of the above traits must be broken.

In order to substantiate the above findings, we estimated the genetic similarity between newly developed lines and their parents using a set of 100 SSR markers equally distributed across the genome and a set of SSRs known to be associated with putative insect resistance gene clusters (data not shown). The chromosomal locations of these clusters were previously reported in two QTL studies using resistant lines D06 and RZ05 as parents (BOHN *et al.*, 2000, PAPST *et al.*, 2001). Accounting for sampling effects, we found significant differences between genetic similarity estimates determined with both SSR sets. A comparison of resistance QTL cluster haplotypes based on SSR markers revealed that early maturing parental lines D06 and D67 contributed the majority of the SSR alleles at the chromosomal cluster regions to their offspring. Due to the high weight put on early maturity in our breeding program and the association between early maturity and ECB resistance, it can be speculated that the observed differences between genetic similarity values were not caused by selection for improved ECB resistance but by selection for early maturity. If the association between ECB resistance and maturity is not caused by pleiotropy but rather by linkage, it will be difficult in conventional breeding to combine the desired alleles for both traits in a single genotype. However, based on graphical genotypes several S_2 families were detected in a QTL population, which showed a high level of ECB resistance associated with early maturity (BOHN *et al.*, 2000). This demonstrates the potential of molecular markers to identify genotypes with the necessary recombination events between tightly linked QTL for ECB resistance and maturity. In addition, this result underlines the importance of random mating within S_1 populations for improving early maturity and ECB resistance at the same time (BOHN *et al.*, 2000).

If breeders could predict the prospects of crosses for inbred line development before producing

and testing lines derived from them in field trials, this would greatly increase the efficiency of breeding programs by concentrating the efforts on the most promising crosses. SCHNELL and UTZ (1975) defined the "usefulness" of a cross for line development as the sum of the population mean of all possible lines derivable from the cross without selection plus the predicted selection gain. Numerous studies on different crops demonstrated that the population mean can be reliably predicted from the midparent value and that the midparent value can be successfully applied for predicting the usefulness of their crosses (for review see UTZ *et al.*, 2001). Therefore, we recommend increasing the changes of selection by rigorous screening of potential parental lines for high ECB resistance before new source populations are developed. However, the extensive line screening performed by SCHULZ *et al.* (1997) and MELCHINGER *et al.* (1998) identified a limited number of lines with ECB resistance. In order to develop new lines combining high ECB resistance with good agronomic performance it seems, therefore, appropriate to initiate recurrent selection programs. These programs will be successful, if lines selected to create the base population will carry different alleles at QTL involved in the inheritance of the ECB resistance or if they combine different resistance mechanisms. Many studies demonstrated that it was possible to improve ECB resistance markedly in the medium term using recurrent selection (PENNY *et al.*, 1967; CHIANG and HUDON, 1973; KLENKE *et al.*, 1986; ANGLADE *et al.*, 1996). Limited information yet is available about the agronomic performance of the lines developed through the recurrent selection process and their fate in commercial breeding programs.

Why make these efforts towards improving host plant resistance in maize, if *Bt*-maize hybrids with an extreme high level of ECB resistance are available? Monogenic resistances are suspected to be overcome quickly by the target pest (METZ *et al.*, 1995). In the future it may, therefore, make sense to place the *Bt* gene in a genetic background that provides an improved level of quantitative resistance against ECB larvae feeding. This combination might help to conserve the effectiveness of the *Bt* genes. In addition, it has to be taken into account that not all farmers do have access to *Bt* hybrids. National regulations might prevent the use of genetically modified organisms (GMOs) or farmers produce maize products for markets that do not accept GMOs. Therefore, non-transgenic host plant resistances will continue to play an important role in securing maize production in the future and the design of new improved breeding strategies are of great importance.

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Comparison of *Bt* maize hybrids with their non-transgenic counterparts and commercial varieties for resistance to European corn borer and for agronomic traits

T. MAGG[1], A. E. MELCHINGER[1], D. KLEIN[1] and M. BOHN[1,2]

[1] Institute for Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, D–70593 Stuttgart, Germany. E-mail: Bohn@pz.uni-hohenheim.de; [2] Corresponding author

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Abstract

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a major pest of maize (*Zea mays* L.) in Central Europe. In order to compare transgenic *Bt* maize hybrids with their non-transgenic counterparts and commercial hybrids, field trials and a laboratory bioassay were conducted. The field experiments were performed at four locations with natural and manual infestation of ECB larvae in 1998 and 1999. Transgenic *Bt* hybrids showed significantly lower means than their corresponding non-transgenic counterparts and commercial hybrids for all resistance traits (damage rating of stalks, number of larvae per plant, and percentage of damaged plants or ears under infestation). *Bt* hybrids containing the *CryIA(b)* gene under the control of green tissue and pollen-specific promoters (event 176) showed a significantly higher percentage of damaged ears than *Bt* hybrids carrying the *CryIA(b)* gene under the control of a constitutive promoter (Mon810). *Bt* and non-*Bt* hybrids showed no significant differences for all agronomic traits, except for plant height under insecticide protection and grain yield reduction under infestation, whereas *Bt* hybrids had significantly lower means than their non-transgenic counterparts and other commercial hybrids. All resistance traits were significantly correlated with grain yield reduction. The laboratory bioassay confirmed the level of antibiosis of *Bt* hybrids against neonate ECB larvae. *Bt* hybrids showed the highest level of ECB resistance and therefore are an attractive method of preventing ECB damage within an integrated pest-management system.

Key words: *Zea mays* — *Ostrinia nubilalis* — *Bacillus thuringiensis* — European corn borer — transgenic maize — larval mortality — bioassay

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a major pest of maize (*Zea mays* L.) in Central Europe and steadily spreads to northern maize-growing regions (Langenbruch and Szewczyk 1995). Moths hatch at the end of June and deposit their eggs on plants at the late whorl stage, before anthesis. ECB larvae tunnel into the stalks and ears causing severe physical damage, which often results in yield losses due to dropped ears or lodged plants. Bohn et al. (1998) reported yield losses of up to 30% in regions with a high natural occurrence of ECB. Damaged plants often show increased susceptibility to secondary infections caused by different pathogens, such as *Fusarium* spp. or *Ustilago maydis* (Lew et al. 1991, Munkvold et al. 1997, 1999). Consequently, expensive insecticide application or biological control measures are necessary to prevent yield losses and secondary infections in regions with high natural occurrence of ECB moths. However, ECB larvae on maize plants are difficult to combat because they are exposed to sprays or antagonists for only a short period before they bore into the plant. A proper monitoring of ECB flight and repeated applications of insecticides are mandatory (Jansens et al. 1997).

Bacillus thuringiensis (*Bt*) has long been used for controlling insect pests as a biologically insecticidal spray. At present, plants obtained by genetic engineering are capable of endogenous production of the toxin. The δ-endotoxin used, which is derived from the soil-borne *Bt* var. *kurstaki* HD-1, is fatal predominantly to insects of Lepidopteran orders such as ECB. The *Bt* gene *CryIA(b)*, encoding for an insecticidal crystalline protein, was one of the first genes to attract interest for use in plant transformation (Meeusen and Warren 1989), followed by the *CryIA(c)* and *Cry9C* genes (Jansens et al. 1997, Archer et al. 2000), which have lethal effects against Lepidopteran species. Gene promoters regulate the tissue-specific and developmental stage-specific expression of the *Bt* gene. Based on the European Council Directive 90/220 and the German Seed Act, only maize hybrids derived from transformation events Mon810 and event 176, both containing the *CryIA(b)* gene, have a restricted license to be used in maize production in Germany. Mon810 uses a gene promoter, which results in a season-long expression of the *Bt* toxin in all plant tissues (Archer et al. 2000). In contrast, event 176 contains two promoters, one regulating *Bt* gene expression exclusively in green plant tissues and the other in the pollen (Koziel et al. 1993, Estruch et al. 1997).

Several studies conducted in the Corn Belt of the USA demonstrated the high level of resistance of *Bt*-transformed maize against ECB (Koziel et al. 1993, Estruch et al. 1997, Jansens et al. 1997, Pilcher et al. 1997, Sagers et al. 1997, Archer et al. 2000). *Bt* maize also showed reduced contamination with *Fusarium* spp. and a lower mycotoxin concentration compared with non-transgenic plants (Munkvold et al. 1997, 1999). However, no studies are available on the effectiveness of *Bt* resistance against ECB in European maize germplasm of early maturity. Based on economic considerations, Bohn et al. (1998) concluded that *Bt* maize should be the most economic ECB control measure under Central European conditions because of its high level of resistance. Therefore, it is important to evaluate the efficiency of *Bt* maize in a Central European climate.

The objectives of this study were to: (1) evaluate the level of ECB resistance and the agronomic performance of early-maturing European *Bt* hybrids, their non-transgenic normal counterparts and recommended commercial hybrids; (2) estimate the correlation between resistance traits and important

agronomic traits; and (3) determine the level of *Bt* resistance in a laboratory bioassay.

Materials and Methods

Plant materials: Two different groups of early-maturing European maize hybrids (maturity < 300 FAO units) were evaluated for ECB resistance in 1998 and 1999. Experiment 1 comprised nine entries: three pairs of hybrids, each consisting of a *Bt* cultivar carrying event 176 and its corresponding non-transgenic counterpart, and three commercial cultivars (for definition of hybrid groups see Table 1). Experiment 2 comprised 17 entries: two pairs of transgenic and non-transgenic hybrids with event 176 and three pairs of transgenic and non-transgenic hybrids with event Mon810, and seven commercial cultivars. The hybrids 'Pactol CB *Bt*', 'Pactol' and 'Symphony' were common to both experiments. The commercial cultivars were taken from the recommended list of maize cultivars in the state of Baden-Württemberg, Germany. The *Bt* hybrids and their non-transgenic counterparts were provided by commercial companies. In this paper, the group containing non-transgenic and commercial hybrids is referred to as non-*Bt* hybrids.

Field trials: Field trials for Experiment 1 were conducted at four locations in 1998 (Auggen, Eckartsweier, Ladenburg, and Trebur) and for Experiment 2 in 1999 (Eckartsweier, Kandel, Ladenburg, and Trebur). All experimental sites are located in the Upper Rhine Valley, the main maize-growing region of Germany. This area has a high natural occurrence of ECB. The experimental design applied at each site was a split plot. Whole-plots consisted of two ECB treatments — one with manual infestation and the other with insecticide protection. In both experiments, subplots were arranged according to an α-design. The subplots comprised the genotypes and consisted of four rows, 4 m long with 26 plants per row in Experiment 1 and 25 plants per row in Experiment 2, with a row spacing of 0.75 m. Plants were machine-sown and manually thinned to a density of 8.7 plants/m^2 in Experiment 1 and 8.5 plants/m^2 in Experiment 2. In the infestation plots, three core rows were infested with neonate ECB larvae; the fourth row was not infested and used to prevent larval migration between plots. In addition, whole-plots were separated by guard plots. Control plots were protected against ECB larvae by a triple application of the insecticide Fastac SC® (alphacypermethrin, BASF AG) starting at the end of June at 10- to 14-day intervals.

Table 1: Characterization of maize hybrids evaluated for European corn borer (ECB) resistance in the two field experiments and in the laboratory bioassay

Cultivar	Characterization	Experiment	
		Field	Laboratory
Experiment 1			
'Cesar *Bt*'	Transgenic, *Bt* 176	1998	1998
'NX6369 *Bt*'	Transgenic, *Bt* 176	1998	1998
'Pactol CB *Bt*'	Transgenic, *Bt* 176	1998, 1999	1998
'Cesar'	Non-*Bt*, Non-transgenic	1998	1998
'NX6368'	Non-*Bt*, Non-transgenic	1998	1998
'Pactol'	Non-*Bt*, Non-transgenic	1998, 1999	1998
'Favola'	Non-*Bt*, Commercial	1998	1998
'Helix'	Non-*Bt*, Commercial	1998	1998
'Symphony'	Non-*Bt*, Commercial	1998, 1999	1998
Experiment 2			
'Mesnil *Bt*'	Transgenic, Mon810	1999	–
'Novelis *Bt*'	Transgenic, Mon810	1999	–
'Pactol CB *Bt*'	Transgenic, *Bt* 176	1998, 1999	–
'Valmont *Bt*'	Transgenic, *Bt* 176	1999	–
'Transal *Bt*'	Transgenic, Mon810	1999	–
'Mesnil'	Non-*Bt*, Non-transgenic	1999	–
'Nobilis'	Non-*Bt*, Non-transgenic	1999	–
'Pactol'	Non-*Bt*, Non-transgenic	1998, 1999	–
'Prelude'	Non-*Bt*, Non-transgenic	1999	–
'Transal'	Non-*Bt*, Non-transgenic	1999	–
'Attribut'	Non-*Bt*, Commercial	1999	–
'Baltimore'	Non-*Bt*, Commercial	1999	–
'Benicia'	Non-*Bt*, Commercial	1999	–
'Clarica'	Non-*Bt*, Commercial	1999	–
'Lob'	Non-*Bt*, Commercial	1999	–
'Prinz'	Non-*Bt*, Commercial	1999	–
'Symphony'	Non-*Bt*, Commercial	1998, 1999	–

Plants were manually infested with ECB larvae to ensure an even infestation level for the entries at all locations, except for Trebur, where no manual infestation was necessary because of the exceptionally high natural population density of ECB (Bohn et al. 1998). The time of manual infestation was synchronized with the natural appearance of ECB moths at the end of June to simulate natural infestation. Egg masses for manual infestation with ECB larvae were purchased from the entomology laboratory of Dr P. Aupinel, Institut National de la Recherche Agronomique (INRA), Le Magneraud, France. For manual infestation, freshly hatched ECB larvae were mixed with corn cob grids and dropped, using a mechanical dispenser (Mihm 1983), into the plant whorl or, at later plant development stages, into the leaf axils. About 20 ECB larvae per plant were applied three times at weekly intervals.

Resistance to larval feeding was measured in the third row of infested plots by longitudinally splitting the stalks and picking the ears shortly before harvest. The following resistance traits were determined: (1) damage rating of stalks (DRS) using a 1–9 damage rating scale (1 for no visible symptoms, 2–3 for broken tassels, 4–8 for stalk breakage above the ear, and 9 for plants broken below the ear), as described by Hudon and Chiang (1991); (2) number of larvae per plant (LAV); (3) percentage of plants displaying ECB feeding damage (PDP); and (4) percentage of damaged ears (PDE) (only in Experiment 2).

Agronomic traits recorded for each plot were as follows. (1) Grain yield in t/ha under protection (GYP) and (2) infestation (GYI), adjusted to 15.5% grain moisture. Only the two centre rows of each plot were harvested. (3) Grain yield reduction (GYR) was calculated for each genotype in per cent by applying the formula $GYR = (1 - GYI/GYP) \times 100$. (4) Grain dry matter in percentage was determined under protection (GDP) and (5) under infestation (GDI) as well as (6) plant height (in cm) under protection (PHP) and (7) under infestation (PHI). The results for PHI are not presented because no significant differences between protected and infested plots were observed.

Laboratory bioassay: In parallel with the field trials in 1998, a laboratory bioassay was performed to investigate the resistance of the nine maize hybrids, based on the mortality of ECB larvae feeding on their leaf tissue. The bioassays were performed essentially as suggested by Wiseman et al. (1981) and Jansens et al. (1997). Leaves above the second or third whorl were harvested from each genotype of the field plots in Eckartsweier. Leaf samples of about 6 cm^2 were sterilized in 70% ethanol and 0.7% sodium hypochlorite and subsequently washed twice in distilled water. One leaf sample was placed into each Petri dish that was covered with a moisturized paperboard lid. Using the mechanical dispenser, the leaf tissue in each Petri dish was infested with an average of 13 neonate larvae, mixed with corn cob grids. The dishes were sealed with parafilm and placed in a climate chamber with a photoperiod of 14 : 10 (day–night) at 22–25°C, 60–70% humidity at day and 14–16°C, 70–80% humidity at night. Larval mortality was evaluated every 2 days (M2, M4, M6) within a 6-day period. Leaves were arranged in Petri dishes according to a randomized complete-block design with five Petri dishes per genotype and replication. The experiment was replicated twice, with a total of 20 Petri dishes per genotype.

Statistical analyses: For each environment the experiment was first analysed according to an α-design. Because efficiency factors were

rather low, incomplete block effects could be ignored. Data for individual plants of each subplot were averaged to obtain a subplot mean for each hybrid and replication. Ordinary analyses of variance for randomized complete-block designs combined across locations were performed separately, with the subplot means of each main plot. In all statistical models, genotypes were considered as fixed effects, whereas other sources of variance were considered as random effects. Linear contrasts were calculated among the following groups: (1) transgenic vs. non-transgenic hybrids (2) transgenic vs. commercial hybrids, and (3) non-transgenic vs. commercial hybrids.

Least significant differences (LSDs) were calculated to test for differences between individual entry means. Phenotypic correlations among traits were calculated based on entry means across locations separately for each experiment using established procedures (Snedecor and Cochran 1980). To assess the proportion of the genetic variance vs. the total phenotypic variance among entries, genetic ratios (GR) on an entry-mean basis were calculated as suggested by Dhillon et al. (1990):

$$GR = \frac{\hat{\phi}_g^2}{\hat{\phi}_g^2 + \hat{\sigma}_{gl}^2/l + \hat{\sigma}_e^2/(l \times r)}$$

where $\hat{\phi}_g^2$ is the genotypic variance among genotypes for a given treatment, $\hat{\sigma}_{gl}^2$ the variance of genotype × location interactions, $\hat{\sigma}_e^2$ the error variance, l the number of locations, and r the number of replications. Both estimates of $\hat{\phi}_g^2$ and GR apply only to the specific group of lines investigated here. All necessary ANOVA computations were performed with the computer package PLABSTAT (Utz 1998), except for the linear contrasts, which were computed with the SAS procedure GLM (SAS Institute 1988).

Results

The *Bt* hybrids showed significantly ($P < 0.05$) lower values for DRS, LAV and PDP than the non-*Bt* hybrids in both experiments (Tables 2 and 3), including the supplementary PDE value added in Experiment 2. For all ECB resistance traits evaluated, means of non-transgenic hybrids showed a larger variation than the means of the commercial hybrids, except for DRS in both experiments. Means for DRS and LAV were significantly ($P < 0.05$) smaller for non-transgenic hybrids than for commercial hybrids. The reverse applied to non-transgenic hybrids, which had significantly ($P < 0.05$) higher LAV and PDP means than the commercial hybrids in Experiment 2. Within the non-*Bt* group, means of PDE showed no significant difference (Table 3). Estimates of GR were high for most traits, except for LAV in both experiments, because of high σ_{gl}^2 estimates.

The *Bt* hybrids and their non-transgenic counterparts were not significantly different for days to anthesis (data not shown), GYP and GDP in the protected plots. In both experiments, the *Bt* hybrids showed significantly ($P < 0.05$) higher GYI values and lower GYR values than the non-*Bt* hybrids. No significant differences between the hybrid groups were detected for GDP and GDI in Experiment 1 (Table 2). In Experiment 2, GDP and GDI were significantly ($P < 0.05$) greater for commercial hybrids than for the *Bt* hybrids, and for GDI the non-transgenic counterparts showed significantly ($P < 0.05$) lower GDI means than the latter. Significant ($P < 0.05$) differences between hybrid group means were found for PHP. In Experiment 1, the highest PHP means were found for non-transgenic hybrids followed by *Bt* hybrids and commercial hybrids. The reverse applied to PHP means in Experiment 2, where 'Mesnil *Bt*' and 'Novelis *Bt*' were significantly ($P < 0.05$) taller than their non-transgenic counterparts under protected conditions (Table 3).

The GYP means were not significantly different between non-transgenic and commercial hybrids. Non-transgenic hybrids had significantly ($P < 0.05$) higher GYI means than commercial hybrids only in Experiment 1. Means for GYR were not significantly different within the non-*Bt* group. Within the non-*Bt* group of Experiment 2, the commercial hybrid 'Lob' and the non-transgenic hybrid 'Prelude' showed

Table 2: Means for the nine maize cultivars of Experiment 1, evaluated across four environments for resistance to European corn borer (ECB) and for agronomic traits

	Resistance traits[1]			Agronomic traits[2]					
Cultivar	DRS (1–9)	LAV (n)	PDP (%)	GYP (t/ha)	GYI (t/ha)	GYR (%)	GDP (%)	GDI (%)	PHP (cm)
Transgenic									
'Cesar *Bt*'	1.28	0.19	31	9.11	9.09	1.5	69.1	68.3	201
'NX6369 *Bt*'	1.15	0.13	24	8.96	8.65	1.8	68.6	68.3	221
'Pactol *Bt*'	1.00	0.10	26	9.05	9.09	−1.2	67.7	68.1	202
Mean	1.14a[3]	0.14a	27a	9.04a	8.94a	0.7a	68.5a	68.2a	208a
Non-transgenic									
'Cesar'	2.89	0.64	84	8.84	7.37	13.9	68.3	69.7	206
'NX6368'	2.25	0.47	76	9.24	8.07	13.2	68.6	69.3	226
'Pactol'	3.04	0.76	90	9.08	8.01	12.8	68.3	68.1	205
Mean	2.73b	0.62b	83b	9.05a	7.82b	13.3b	68.4a	69.0a	212b
Commercial									
'Favola'	3.69	0.91	97	8.61	7.74	10.6	64.5	66.2	198
'Helix'	2.90	0.96	86	8.01	7.01	11.0	71.4	72.2	208
'Symphony'	4.44	0.81	87	7.53	6.11	19.8	71.4	72.4	196
Mean	3.68c	0.89c	90b	8.05a	6.95c	13.8b	69.1a	70.3a	200c
Total mean	2.51	0.55	67	8.72	7.88	9.3	68.7	69.2	207
LSD 5%	1.21	0.61	17	0.83	0.83	10.5	1.46	1.36	8.56
Genetic ratio (GR)	0.88	0.62	0.96	0.76	0.92	0.73	0.94	0.95	0.92

[1] DRS = damage rating of stalks; LAV = number of larvae per plant; PDP = percentage of damaged plants.
[2] GYP = grain yield in protected plots; GYI = grain yield in infested plots; GYR = grain yield reduction; GDP = grain dry matter content in protected plots; GDI = grain dry matter content in infested plots; PHP = plant height in protected plots.
[3] Means with different letters in columns are significantly different at $P = 0.05$.

Table 3: Means for the 17 maize cultivars of Experiment 2, evaluated across four environments for resistance to European corn borer (ECB) and for agronomic traits

	Resistance traits[1]				Agronomic traits[2]					
Cultivar	DRS (1–9)	LAV (n)	PDP (%)	PDE (%)	GYP (t/ha)	GYI (t/ha)	GYR (%)	GDP (%)	GDI (%)	PHP (cm)
Transgenic										
'Mesnil *Bt*'	1.84	0.09	21	1	11.2	10.8	3.5	73.0	73.5	235
'Novelis *Bt*'	1.48	0.08	18	1	11.7	11.5	1.4	71.0	71.7	222
'Pactol CB *Bt*'	1.42	0.12	18	4	11.7	11.7	−0.2	75.9	75.4	225
'Valmont *Bt*'	1.65	0.11	26	12	12.8	12.5	2.4	74.8	75.5	234
'Transal *Bt*'	2.34	0.09	20	2	11.6	11.6	1.4	74.7	74.8	236
Mean	1.75a[3]	0.10a	21a	4a	11.8a	11.6a	1.7a	73.9a	74.2a	230a
Non-transgenic										
'Mesnil'	2.84	0.78	78	34	11.9	10.1	15.8	73.3	74.2	221
'Nobilis'	3.97	1.03	98	58	11.4	9.1	20.0	70.8	72.6	203
'Pactol'	3.36	0.79	78	16	11.2	9.9	12.0	75.2	75.4	225
'Prelude'	3.06	0.47	74	22	12.1	11.1	8.8	74.4	75.0	228
'Transal'	3.77	0.83	82	9	11.6	10.1	13.0	75.5	76.5	242
Mean	3.40b	0.78b	82b	28b	11.6a	10.1b	13.9b	73.8a	74.8b	224b
Commercial										
'Attribut'	3.57	0.71	71	36	11.6	9.8	16.3	72.8	75.2	244
'Baltimore'	3.44	0.50	82	27	12.1	10.3	14.3	71.9	74.2	244
'Benicia'	3.56	0.56	68	7	12.9	11.0	16.4	76.3	76.5	264
'Clarica'	3.38	0.62	79	24	12.2	10.4	15.8	76.8	77.4	248
'Lob'	2.72	0.62	65	10	11.7	10.7	8.6	76.1	76.2	255
'Prinz'	3.71	0.60	75	24	11.0	10.0	9.0	76.2	77.7	227
'Symphony'	4.34	0.59	87	49	10.2	7.9	21.8	77.7	78.6	222
Mean	3.53b	0.60c	75c	25b	11.7a	10.0b	14.6b	75.4b	76.5c	243c
Total mean	2.97	0.50	61	20	11.7	10.5	10.6	74.5	75.3	234
LSD 5%	0.86	0.55	19	18	0.10	0.14	10.4	1.28	1.07	8.87
Genetic ratio (GR)	0.89	0.60	0.94	0.86	0.69	0.79	0.72	0.95	0.96	0.96

[1] DRS = damage rating of stalks; LAV = number of larvae per plant; PDP = percentage of damaged plants; PDE = percentage of damaged ears.
[2] GYP = grain yield in protected plots; GYI = grain yield in infested plots; GYR = grain yield reduction; GDP = grain dry matter content in protected plots; GDI = grain dry matter content in infested plots; PHP = plant height in protected plots.
[3] Means with different letters in columns are significantly different at P = 0.05.

the lowest GYR caused by larval feeding, whereas the commercial hybrid 'Symphony' and the non-transgenic hybrid 'Nobilis' were highly susceptible to ECB and showed substantial yield losses. In Experiment 2, the commercial hybrids showed significantly ($P < 0.05$) higher GDP and GDI means than the non-transgenic hybrids. Non-transgenic hybrids were significantly ($P < 0.05$) taller than commercial hybrids in Experiment 1 but significantly ($P < 0.05$) smaller in Experiment 2. Estimates of GR were moderate to high for most agronomic traits in both experiments except for GYR in Experiment 1.

Resistance traits were significantly ($P < 0.01$) correlated with each other ($0.69 \leq r_p \leq 0.95$) in both experiments (Table 4). Most correlations among agronomic traits were not significantly different from zero. Significant ($P < 0.05$) correlations of medium size were found between GYP and PHP in Experiment 2, as well as between GYI and GDI in Experiment 1. GYI and GYR showed significantly ($P < 0.01$) negative correlations ($-0.74 \geq r_p \geq -0.91$) with all resistance traits in both experiments. The correlation coefficient between GDI and DRS was significant ($P < 0.05$) only in Experiment 2.

In the laboratory bioassay, mortality of neonate larvae increased with increasing exposure time of the larvae to the leaf samples of the hybrids. After 2 days (M2) of exposure, no significant differences in larval mortality between hybrid groups were observed. Extending the exposure of neonates to maize leaf samples for a further 2 days (M4) and 4 days (M6) increased the larval mortality steadily and resulted in significant ($P < 0.01$) differences between hybrids and a clear separation of mortality between the three hybrid groups, with the greatest ($P < 0.05$) larval mortality observed for the *Bt* hybrids (Table 5).

Discussion

ECB resistance of *Bt* and non-*Bt* maize hybrids

In both experiments, the *Bt* maize hybrids evaluated demonstrated their superiority with regard to the control of ECB larval feeding. The larvae caused nearly no grain yield reduction in transgenic maize hybrids. The number of larvae per plant, damaged stalks and damaged ears were greatly reduced in *Bt* maize compared with their non-transgenic counterparts and commercial hybrids. Even though *Bt*-transformed maize was highly effective in controlling ECB larval attacks, the larvae damaged the transgenic plants to some extent and, on average, 0.08–0.19 larvae per plant survived the consumption of *Bt* maize plant tissue. There are three possible explanations for this observation: (1) ECB larvae have to feed on *Bt* maize and subsequently damage the plants to take up the *Bt* toxins before they die; (2) tissue- and time-specific expression of the toxin, depending on the used *Bt* event may allow survival of later instar ECB larvae (Koziel et al. 1993, Estruch et al. 1997); (3) ECB larvae may migrate from susceptible plants to *Bt* maize genotypes.

With regard to the first explanation, Orr and Landis (1997) showed that the level of non-preference, measured as the

Table 4: Phenotypic correlation coefficients for maize hybrids evaluated in Experiment 1 and 2

	Resistance traits[1]			Agronomic traits[2]					
	PDP (%)	LAV (n)	DRS (1–9)	GYR (%)	GDI (%)	GYI (t/ha)	PHP (cm)	GDP (%)	GYP (t/ha)
Experiment 1									
GDP									−0.56
PHP								0.09	0.46
GYI							0.45	−0.50	0.86**
GDI						−0.73*	−0.09	0.95**	−0.74*
GYR					−0.53	0.89**	−0.29	−0.29	0.57
DRS				−0.91**	0.39	−0.90**	−0.63	0.11	−0.71*
LAV			0.90**	−0.81**	0.36	−0.81**	−0.53	0.08	−0.62
PDP	–	0.95**	0.90**	−0.89**	0.28	−0.77*	−0.49	0.00	−0.45
Experiment 2									
GDP									−0.09
PHP								0.38	0.52*
GYI							0.27	−0.10	0.68**
GDI						−0.33	0.38	0.93**	−0.17
GYR					−0.33	0.84**	0.00	−0.08	0.18
DRS				−0.90**	0.49*	−0.84**	0.04	0.23	−0.30
LAV			0.84**	−0.81**	0.24	−0.74**	−0.10	0.03	−0.18
PDP		0.93**	0.92**	−0.91**	0.37	−0.78**	−0.04	0.12	−0.18
PDE	0.75**	0.69**	0.71**	−0.81**	0.12	−0.78**	−0.45	−0.14	−0.36

*,** Significant at P = 0.05 and P = 0.01, respectively.
[1] DRS = Damage rating of stalks; LAV = number of larvae per plant; PDP = percentage of damaged plants; PDE = percentage of damaged ears.
[2] GYP = Grain yield in protected plots; GYI = grain yield in infested plots; GYR = grain yield reduction; GDP = grain dry matter content of in protected plots; GDI = grain dry matter content in infested plots; PHP = plant height in protected plots.

Table 5: Means of mortality of neonate European corn borer (ECB) larvae in the laboratory bioassay for hybrids of Experiment 1

	Mortality[1,2]		
Cultivar	M2 (%)	M4 (%)	M6 (%)
Transgenic			
'Cesar *Bt*'	15.1	83.6	97.4
'NX6369 *Bt*'	17.7	83.3	97.0
'Pactol CB *Bt*'	14.5	86.8	97.9
Mean	15.8bc	84.6a	97.4a
Non-transgenic			
'Cesar'	12.1	51.9	78.0
'NX6368'	7.0	41.0	64.6
'Pactol'	18.7	58.3	79.9
Mean	12.6ab	50.4b	74.2b
Commercial			
'Favola'	14.2	54.1	81.4
'Helix'	27.4	66.5	89.5
'Symphony'	22.2	63.7	83.0
Mean	21.3c	61.4c	84.6c
Total mean	16.6	65.5	85.4
LSD 5%	24.7	18.1	11.4
Genetic ratio (GR)	0	88.2	90.0

[1] M2, M4 and M6 = Mortality of neonates after 2, 4 and 6 days of exposure to leaf material, respectively.
[2] Means with different letters in columns are significantly different at P = 0.05.

number of egg masses per plant (oviposition) was not significantly different between *Bt* maize and their non-transgenic counterparts. Therefore, the damaged stalks, and also the leaves, indicate that ECB larvae feed on the *Bt* plants to take up the *Bt* toxin. With regard to the second explanation, Onstad and Gould (1998) expressed the concern that hybrids with a declining endotoxin titre are more likely to allow ECB survival and development of resistance to *Bt* may be accelerated. Archer et al. (2000) evaluated four different events of *Bt* maize from the elite US germplasm. They showed that event 176 provided a less effective control of second generation ECB larvae, i.e. *Bt* hybrids were similar to non-*Bt* hybrids under US conditions and larvae survived the initiated over-wintering experiment. This means that some larvae might survive the relatively low doses of toxin expressed by *Bt* hybrids and therefore thinning effects of *Bt* toxin could be observed in *Bt* hybrids in later development stages. Furthermore about 2% of the plants in *Bt* hybrids do not carry the *Bt* gene (N. Mülleder, Monsanto Agrar Deutschland GmbH, pers. comm.). ECB larvae migration can be observed (Ross and Ostlie 1990) as a result of the small field plots, even though a guard row was planted to prevent migration between adjacent plots. However, based on the results of our study it can be concluded that both *Bt* events provided an effective ECB control measure under central European conditions.

Among the early maturing elite European non-*Bt* hybrids, 'Lob' and 'Prelude' showed the smallest grain yield reduction caused by larval feeding, whereas 'Symphony' and 'Nobilis' were highly susceptible to ECB and showed substantial yield losses. These results are in agreement with the findings of various studies (Schulz et al. 1997, Kreps et al. 1998, Melchinger et al. 1998), in which a substantial genetic variation for insect resistance against ECB in the early maturing European maize germplasm was reported.

Consequences for a resistance management system

Effective resistance management is essential to prevent the rapid development of ECB individuals resistant to the *Bt*

toxin. One of the major strategies of *Bt* resistance management is the 'refuge plus high-dose' strategy. Here, specific assumptions must be fulfilled to guarantee success in conserving the effectiveness of the monogenic *Bt* resistance. (1) Plant tissue must be highly toxic during the whole growing season to ensure that insect genotypes heterozygous for the *Bt* resistance are killed (Roush and McKenzie 1987). (2) The resistance allele must be rare ($p < 10^{-3}$) in the insect population, so that there will be only a few homozygous survivors ($p^2 < 10^{-6}$) (Roush 1994). However, it is unclear how frequently *Bt* resistance alleles do occur in ECB populations (Bolin et al. 1999). (3) *Bt* plots should be interspersed with an arrangement of non-toxic refuges, so that resistant homozygotes will mate randomly or preferentially with susceptible homozygotes, producing heterozygous progeny that cannot survive feeding on the *Bt* crop (Tabashnik 1994, Alstad and Andow 1995, Andow et al. 1998). In our field trial, small plots were used for determining the resistance of maize genotypes carrying the *Bt* gene. The experiments were not designed to validate the assumptions concerning the refuge plus high-dose strategy. However, the results of 0.08–0.19 larvae per plant and a percentage of damaged stalks ranging from 18–31% for *Bt* hybrids do question the first assumption underlying the refuge plus high-dose strategy. If a high-dose strategy cannot be achieved, and a small fraction of homozygous susceptible and heterozygous ECB neonates would survive feeding on *Bt* hybrids, resistance can develop in 10 33% of the time required under the assumption that a successful high-dose strategy would kill all heterozygous neonates (Onstad and Gould 1998). Ostlie et al. (1997) emphasized that it is unclear how high a high dose needs to be in order to qualify for the high-dose strategy. Gould (1994) suggested the use of *Bt* toxin doses that are 25 times higher than the LD_{99} of susceptible insect strains, which should ensure killing all or most heterozygotes.

It could also be possible that maize ears serve as a refuge for ECB larvae after anthesis in transgenic hybrids expressing low or no levels of toxins in the ears. In our study, a clear trend was found towards higher values of damaged ears for hybrids expressing event 176 than hybrids with event Mon810 (Table 3). In addition, surviving larvae were observed in damaged maize ears, especially in hybrids containing event 176, with fewer in hybrids containing Mon810. However, the effects of surviving larvae on the adaptation of ECB to *Bt* maize remains unclear, especially regarding the ear shelter as refuge (Onstad and Gould 1998). Therefore, an escorting trial monitoring of *Bt* hybrids and the respective ECB populations seems to be necessary.

Resistance mechanisms

ECB occurs mainly with one generation per year in Central Europe. This generation is comparable to damage caused by second-generation ECB in the US Corn Belt. In contrast to first-generation ECB leaf feeding resistance, which is mainly based on the concentration of the chemical compound 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) in the leaves, second-generation ECB resistance is determined by the content of detergent fibre, cellulose, lignin, and biogenic silica. These components contribute to an increased tissue toughness. In contrast to the monogenic *Bt* resistance, genetic studies (Bergvinson et al. 1994, Bohn et al. 1999) showed that multiple genes are involved in the inheritance of the host plant resistance against ECB in maize. The host plant resistance is based on antibiosis, tolerance and non-preference (Painter 1968, Panda and Khush 1995).

The relative importance of antibiosis and tolerance components of resistance against ECB can be evaluated by linear regression of grain yield reduction on damage rating of stalks (Ortega et al. 1980). For the *Bt* hybrids, a tight association between grain yield reduction and damage rating of stalks ($r_p \leq -0.90$) was detected, indicating that antibiosis is the main resistance mechanism for maize hybrids carrying the *Bt* gene. In contrast to the *Bt* hybrids, non-*Bt* hybrids showed only a moderate negative correlation between grain yield reduction and damage rating of stalks ($r_p < -0.64$). This is in agreement with findings of Kreps et al. (1998), who also reported a moderate negative correlation between the two traits in conventional breeding material. These results indicate that tolerance and antibiosis both contribute to resistance in non-*Bt* hybrids. To ensure a uniform infestation level of ECB larvae, maize plants were manually infested so that it was not possible to obtain information about non-preference.

Laboratory bioassay

In general, laboratory bioassays can be used to determine the level of antibiosis or non-preference present in maize genotypes. The laboratory bioassay employed in this investigation was applied to differentiate between *Bt* and non-*Bt* hybrids. Significant ($P < 0.01$) differences were found for mortality of neonate larvae between transgenic and non-*Bt* hybrids after feeding leaf material for 4–6 days. The increased larval mortality after 2 days of feeding on transgenic leaf material resulted from the direct *Bt* antibiosis of transgenic hybrids. Several studies evaluated the impact of *Bt* toxins on the mortality of different instars of ECB larvae. Sagers et al. (1997) reported that neonate ECB larvae took only a few bites of the transgenic tissues before they stopped feeding; the insects were dead within 24 h, provided that the LC_{50} for *CryIA(b)* was about 20–30 ng/g diet. Koziel et al. (1993) found a good correlation between the level of mortality after 48 h and the level of *CryIA(b)* toxin detected by enzyme-linked immunosorbent assay in leaf tissue. Gould (1994), Jansens et al. (1997) and Pilcher et al. (1997) also demonstrated that 1st instar larvae have a higher sensitivity to *Bt* toxin-expressing events than later instar ECB larvae. These results are in agreement with the present findings under field conditions and may also explain the relatively high recovery rate of later instar larvae in the different tissues of the *Bt* transformation events evaluated here. In conclusion, the laboratory bioassay used was effective for distinguishing between *Bt* and non-*Bt* genotypes.

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Relationship between European corn borer resistance and concentration of mycotoxins produced by *Fusarium* spp. in grains of transgenic *Bt* maize hybrids, their isogenic counterparts, and commercial varieties

T. MAGG[1], A. E. MELCHINGER[1], D. KLEIN[1] and M. BOHN[1,2]

[1] Institute for Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, D–70593 Stuttgart, Germany; [2] Corresponding author. E-mail: mbohn@uni-hohenheim.de

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Abstract

The European corn borer (ECB), *Ostrinia nubilalis* Hb., is a major pest of maize in central Europe and promotes the infection of maize with *Fusarium* spp. In this study, transgenic *Bt* maize hybrids were compared with their isogenic counterparts, and with commercial hybrids from the recommended list with regard to their level of ECB resistance and their concentration of deoxynivalenol (DON), its 15-acetyl (15-A-DON) and 3-acetyl (3-A-DON) derivatives, nivalenol (NIV), fusarenon-X (FUS-X), fumonisins (FUM), and zearalenon (ZEN) in harvested grains. The field experiments were performed in Germany at four locations in 1999 and at five locations in 2000. Transgenic *Bt* hybrids showed significantly lower means than their corresponding isogenic counterparts and than commercial hybrids for all resistance traits: damage rating of stalks, number of larvae per plant, number of larvae per ear, and percentage of damaged plants or ears under infestation. Among all mycotoxins analysed, DON consistently showed the highest concentration across all year × location combinations. Mycotoxin concentrations varied significantly between locations, years and genotypes, whereas mycotoxin concentrations were not significantly different between infested and protected plots. Associations between ECB resistance traits and mycotoxin concentrations were not consistent across years. It is concluded that under central European conditions, the use of *Bt* maize hybrids will only slightly reduce the contamination of maize kernels with mycotoxins produced by *Fusarium* spp.

Key words: *Zea mays* — *Ostrinia nubilalis* — *Bacillus thuringiensis* — *Fusarium* spp. — mycotoxin concentration — transgenic maize

The European corn borer (ECB), *Ostrinia nubilalis* Hb., is a major pest of maize in central Europe. Larvae of this insect tunnel into the stalks and ears, causing severe physical damage, which often results in yield losses due to dropped ears or lodged plants. Yield losses may reach up to 30% in regions with a high natural occurrence of ECB (Bohn et al. 1998). The univoltine ECB causes severe damage during the reproductive stages of maize under central European conditions. This damage is comparable to the second ECB generation under Corn Belt conditions of the USA. In general, ECB larvae are controlled by insecticides or biological means, e.g. through the use of *Trichogramma evanescens*, but recently transgenic maize cultivars, carrying the *Bt* gene, proved to bear a highly effective alternative control method (Koziel et al. 1993, Estruch et al. 1997, Jansens et al. 1997, Pilcher et al. 1997, Sagers et al. 1997, Archer et al. 2000, Magg et al. 2001).

Plants with injuries caused by feeding larvae often show increased susceptibility to secondary infections due to different pathogens such as *Fusarium* spp. or *Ustilago maydis* (Jarvis et al. 1984, Lew et al. 1991, Munkvold et al. 1997, 1999). In central Europe, the most common *Fusarium* species are *F. graminearum*, *F. culmorum*, *F. subglutinans*, *F. avenaceum* and *F. moniliforme* (Lew 1993, Bottalico 1998), causing stalk, ear and root rot of maize. In addition, *Fusarium* spp. produce mycotoxins with detrimental effects on humans and animals (IARC 1993) such as type B trichothecenes, including DON, its 15-A-DON and 3-A-DON derivatives, NIV and FUS-X. ZEN, FUM, moniliformin, and beauvericin are also of importance.

It is hypothesized that ECB larvae are vectors for *Fusarium* spp. by causing entry wounds and carrying fungal inoculum from the plant surfaces into the plant itself. Furthermore, Jarvis et al. (1984) reported a close association between susceptibility of maize hybrids to second ECB generation damage and the appearance of stalk rot. Further studies showed that specific *Fusarium* strains were favoured by ECB larvae feeding, whereas the frequency of other *Fusarium* strains was considerably reduced (Lew 1993). Ajanga and Hillocks (2000) found a strong association between cob rot incidence and maize stalk damage caused by *Busseola fusca* in Kenya. They assumed that this stalk borer species predisposes the ears to fungal infections. In addition, *Bt* maize hybrids, expressing the *Bt* toxin in the ears, were less prone to *Fusarium*-caused ear rots and contained less FUM than their isogenic counterparts in the Corn Belt of the USA (Munkvold et al. 1997, 1999).

The overall goal of this study was to evaluate the association between ECB resistance and mycotoxin concentration in early maturing European maize germplasm. The objectives were: (1) to determine the level of ECB resistance in early-maturing European *Bt* hybrids, their isogenic counterparts, recommended commercial cultivars, and experimental hybrids; (2) to measure the mycotoxin concentration in these genotypes; and (3) to evaluate the association between ECB resistance and mycotoxin concentrations.

Materials and Methods

Plant materials: Two different groups of early-maturing European maize, *Zea mays* L., hybrids (maturity < 300 FAO units) were

evaluated for ECB resistance and mycotoxin concentration in 1999 and 2000.

Experiment 1 is identical to experiment 2 described by Magg et al. (2001) and comprised 17 entries: two pairs of hybrids, each consisting of a *Bt* cultivar carrying event 176 and its corresponding isogenic counterpart, three pairs of transgenic and isogenic hybrids with event Mon810 and seven commercial cultivars (Table 1).

Experiment 2 comprised 15 entries: two pairs of hybrids, each consisting of a *Bt* cultivar carrying event 176 and its corresponding isogenic counterpart, two pairs of transgenic and isogenic hybrids with event Mon810, three commercial cultivars, and four experimental hybrids from a breeding programme designed to improve ECB feeding resistance by conventional selection methods (Table 2).

The transgenic and isogenic hybrids as well the commercial cultivars 'Symphony', 'Attribut', and 'Clarica' were common to both experiments. The latter were taken from the recommended list of maize cultivars in the state of Baden-Württemberg. The *Bt* hybrids and their isogenic counterparts were provided by Monsanto Agrar Deutschland GmbH (Düsseldorf, Germany) and Syngenta Seeds GmbH (Bad Salzuften, Germany). In this paper, the group containing isogenic and commercial hybrids is referred to as non-*Bt* hybrids.

In both experiments, *Bt* hybrids carried the *CryIA(b)* gene. Transformation event Mon810 utilizes a gene promoter, which results in a season-long expression of the *Bt* toxin in all plant tissues (Archer et al. 2000). In contrast, transformation event 176 contains two promoters regulating *Bt* gene expression in green plant tissues and the pollen, respectively (Koziel et al. 1993, Estruch et al. 1997).

Field trials: Field trials for experiment 1 were conducted at four locations in 1999 (Eckartsweier, Kandel, Trebur and Ladenburg) and at five locations for experiment 2 in 2000 (Eckartsweier, Kandel, Trebur, Freising and Ingolstadt). All experimental sites are located in the Upper Rhine Valley except for Freising and Ingolstadt, which are located in Bavaria. All sites are located in the main maize-growing regions of Germany with high natural occurrence of ECB. The experimental design applied at each site was a split plot with two replications. Whole plots consisted of two ECB treatments, one with manual or natural ECB infestation and the other with insecticide protection. In both experiments, subplots were arranged according to an α-design. The subplots comprised the genotypes and consisted of four rows, 4 m long with 25 plants and a row spacing of 0.75 m. Plants were machine-sown and manually thinned to a density of 8.5 plants/m^2. In the infestation plots, three core rows were infested with neonate ECB larvae; the 4th row was not infested and was used to prevent larval migration between plots. In addition, whole plots were separated by guard plots. Insecticide-protected plots were guarded against ECB larvae by a triple application of the insecticide Fastac SC® (alphacypermethrin, BASF AG, Ludwigshafen, Germany) starting at the end of June at 10- and 14-day intervals. Plants of the infested plots were manually infested with ECB larvae to ensure an even infestation level for the entries at all locations, except for Trebur, where no manual infestation was necessary because of the exceptionally high natural population density of ECB (Bohn et al. 1998). The time of manual infestation was synchronized with the natural appearance of ECB moths at the end of June to simulate natural infestation. Egg masses for manual infestation with ECB larvae were purchased from the entomology laboratory of Dr P. Aupinel, Institut National de la Recherche Agronomique (INRA), Le Magneraud, France. For manual infestation, freshly hatched ECB larvae were mixed with corn cob grids and dropped, using a mechanical dispenser (Mihm 1983) into the plant whorl or, at later plant development stages, into the leaf axils. About 20 ECB larvae were applied three times at weekly intervals.

ECB resistance traits: Resistance to larval feeding was measured in the third row of infested plots by splitting the stalks longitudinally and collecting the ears shortly before harvest. The following resistance traits were determined: (1) damage rating of stalks (DRS) using a 1–9 damage rating scale (1 for no visible symptoms, 2–3 for broken tassels, 4–8 for stalk breakage above the ear and 9 for plants broken below the ear), as described by Hudon and Chiang (1991); and (2) number of larvae per plant (LPP); (3) percentage of plants displaying ECB feeding damage (PDP); and (4) percentage of damaged ears (PDE); (5) number of larvae per ear (LPE) was only evaluated in experiment 2.

Analysis of mycotoxins: In experiment 1, *Fusarium* mycotoxin concentrations were determined for subplots in the infested whole plots. Here, a random grain sample of 1 kg per plot was taken. A subsample of 100 g was ground and meal was taken for the subsequent analyses of mycotoxin concentrations. The analytical procedure was performed by liquid chromatography (LC) with diode array detection and gas chromatography (GC) with electron capture detection (ECD) in order to determine concentration of DON, 15-A-DON, 3-A-DON, NIV and ZEN. The limits of detection were 30 μg/kg for DON, 15-A-DON, 3-A-DON and NIV, and 500 μg/kg for ZEN (Walker and Meier 1998). For experiment 2 representative grain samples were taken from each subplot of the infested and the protected whole plot. The concentrations of DON, 3-A-DON, 15-A-DON, NIV and FUS-X were determined using Mycosep-Clean up and GC–ECD validation. FUM concentration was assessed employing IAS-Clean up and *o*-phthaldialdehyd with high-pressure liquid chromatography (HPLC) fluorescence detection. The limits of detection for the trichothecenes and FUM varied from 60 to 109 μg/kg (Schuhmacher et al. 1997, Weingärtner et al. 1997, Solfrizzo et al. 2001). The analyses of mycotoxin concentrations for experiment 1 were performed by the Institute of Phytomedicine at the University of Hohenheim, Germany, and for experiment 2 by the Institute for Agrobiotechnology at Tulln, Austria.

Statistical analyses: Data from individual plants of each subplot were averaged to obtain a subplot mean for each hybrid and replication. For all ECB resistance traits an ordinary analysis of variance for a randomized complete block design (RCBD) combined across locations was performed with the subplot means, because all resistance traits were evaluated exclusively in the ECB-infested whole plots. The same procedure was used for the analyses of mycotoxin data obtained in experiment 1. The mycotoxin data of experiment 2 were analysed as a split-plot design across locations. Because the residual error terms of the mycotoxin data did not follow a normal distribution, the data were log transformed in both experiments (Grimm 1960). In all statistical models, genotypes and ECB treatments were considered as fixed effects, whereas the locations and the genotype × location interactions were considered as random effects. The sums of squares for genotypes were subdivided to test for linear contrasts among the following groups: (1) transgenic vs. isogenic hybrids; (2) transgenic vs. commercial hybrids; and (3) isogenic vs. commercial hybrids. In experiment 2, the group of commercial hybrids also included the experimental hybrids. Least significant differences (LSDs) were calculated to test for differences between individual entry means of ECB resistance traits.

Phenotypic correlations among traits were calculated based on entry means across locations separately for each experiment using established procedures (Snedecor and Cochran 1980). To assess the proportion of the genetic variance vs. the total phenotypic variance among entries, genetic ratios (GRs) on an entry-mean basis were calculated as suggested by Dhillon et al. (1990):

$$GR = \frac{\hat{\phi}_g^2}{\hat{\phi}_g^2 + \hat{\sigma}_{gl}^2/l + \hat{\sigma}_e^2/(l \times r)}$$

where $\hat{\phi}_g^2$ is the genotypic variance among genotypes for a given treatment, $\hat{\sigma}_{gl}^2$ the variance of genotype × location interactions, $\hat{\sigma}_e^2$ the error variance, l the number of locations, and r the number of replications. The estimates of ϕ_g^2 and GR apply only to the specific group of genotypes investigated here. All necessary ANOVA computations were performed with the software package PLABSTAT (Utz 1998). Contrasts were computed with the procedure PROC GLM for mixed models as implemented in SAS (SAS Institute 1988).

Results

ECB resistance

The *Bt* hybrids showed significantly ($P < 0.05$) lower values for DRS, LPP, LPE, PDP and PDE than the non-*Bt* hybrids in both experiments (Tables 1 and 2). For all evaluated ECB resistance traits, the means of non-*Bt* hybrids showed a larger variation than the means of the *Bt* hybrids. Means for LPP and PDP were significantly ($P < 0.05$) smaller for commercial hybrids than for isogenic hybrids in experiment 1 and the reverse applied for PDE in experiment 2. Estimates of GR were high for most traits, except for LPP in experiment 1 and LPE in experiment 2.

Mycotoxin concentrations

Experiment 1

For DON, 58.8% of the samples showed a detectable concentration, whereas for NIV only 9.6% of the samples were above the detection limit (Table 1). For ZEN only one entry was detected with a concentration above the detection limit (data not shown). Significant ($P < 0.05$) differences between locations as well as significant ($P < 0.01$) differences between means of genotypes were identified only for DON and for 15-A-DON (Tables 1 and 3). For both toxins, *Bt* hybrids showed significantly ($P < 0.05$) lower concentrations than non-*Bt* hybrids. In contrast, commercial hybrids showed significantly ($P < 0.05$) higher 3-A-DON concentrations than *Bt* hybrids and isogenic hybrids. No significant differences between hybrid groups were detected for NIV. The GR was low to moderate and ranged from 0 for NIV to 0.77 for 15-A-DON (Table 1).

Experiment 2

For DON, 82% of the samples showed a detectable concentration, whereas for 3-A-DON only 9% of the samples were above the detection limit (Table 2). Significant ($P < 0.05$) differences between group means were obtained for DON, 15-A-DON, 3-A-DON, and NIV. *Bt* hybrids displayed significantly ($P < 0.05$) lower DON, 3-A-DON, and NIV concentrations than isogenic hybrids. DON and 15-A-DON concentrations of commercial hybrids were significantly ($P < 0.05$) lower than DON and 15-A-DON concentrations of isogenic hybrids (Table 2). Significant ($P < 0.01$) differences between locations were detected for DON, 15-A-DON, FUS-X and FUM (Table 3). Differences between protected and ECB-infested treatments were not significant across locations (Table 3). Values of GR were low to moderate and ranged from 0.16 for 3-A-DON to 0.65 for DON (Table 2).

Phenotypic correlations

Among ECB resistance traits, significant ($P < 0.05$) correlations were found ($0.58 \leq r_p \leq 0.93$) in both experiments (data not shown). Among mycotoxin concentrations, most correlations were positive but not significant. In both experiments, DON was significantly ($P < 0.01$) correlated with 15-A-DON ($0.83 \leq r_p \leq 0.86$).

In experiment 1, ECB resistance traits and mycotoxin concentrations were positively associated. 15-A-DON showed significant ($P < 0.01$) correlations with LPP and PDP ($0.67 \leq r_p \leq 0.70$), whereas NIV was significantly ($P < 0.01$) associated with PDE ($r_p = 0.61$). In experiment 2, no correlations were significant and they ranged from negative to positive values ($-0.31 \leq r_p \leq 0.58$). In order to avoid group effects caused by the highly resistant *Bt* hybrids, phenotypic correlations were also calculated only with entry means of non-*Bt* hybrids as displayed in Figs 1 and 2. Among non-*Bt* hybrids, most correlation coefficients between ECB resistance traits and mycotoxin concentrations were not significantly different from zero, except for the correlations of NIV with DRS and PDE in experiment 1 and between DON and DRS, as well as between 15-A-DON and DRS in experiment 2.

Discussion

ECB resistance of *Bt* vs. non-*Bt* genotypes

In both experiments, the *Bt* maize hybrids evaluated were superior with regard to control of ECB larval feeding. The number of larvae per plant, damaged stalks, damaged ears and larvae per ear were substantially reduced in *Bt* maize in comparison with their isogenic counterparts and commercial hybrids. The differences between hybrids carrying the transformation event 176 and hybrids carrying the transformation event Mon810 were not significant. This is in contrast to the investigations of Archer et al. (2000) with elite USA germplasm, who found event 176 to be less effective in the control of second-generation ECB larvae than three other *Bt* events.

In experiment 2, only a small number of larvae survived initial feeding on the leaves of *Bt* hybrids and were capable of boring into the stalks or feeding on the ears. Therefore, differences between event 176 and Mon810 were not significant. However, in experiment 1, the severity of ECB larval feeding was much higher, resulting in more larvae per plant and a higher percentage of damaged ears for maize hybrids containing event 176 than for hybrids carrying Mon810 (Magg et al. 2001). In general, natural infestation levels are low in central Europe, resulting in a low percentage of damaged ears. Therefore, the absence of *Bt* toxin in ears of maize hybrids containing event 176 is only a minor disadvantage under central European cultivation and ECB infestation conditions.

Mycotoxin concentration

For all mycotoxins evaluated in both experiments, concentrations were substantially lower in 1999 than in 2000. In addition, a highly significant ($P < 0.01$) environmental variance was found for most mycotoxin concentrations. In 2000, average DON and 15-A-DON concentrations were much higher in the experimental sites located in the Rhine valley than in those located in Bavaria. All other mycotoxin concentrations were close to the detection limit, except for FUM in Kandel and FUS-X in Freising. These results are presumably attributable to the different climatic conditions with regard to precipitation and temperatures in the Rhine valley and Bavaria but may also be due to regional differences between the species and strain composition of the *Fusarium* populations.

ECB vs. protected plots

The mycotoxin concentrations were not significantly different between protected and ECB-infested plots. This indicates that the concentration of mycotoxins is largely independent of ECB larval feeding. In agreement with this observation, the mycotoxin concentrations were considerably lower in experiment 1 than in experiment 2, although the incidence of ECB damage

Table 2: Means for the 15 maize cultivars of experiment 2 evaluated across five locations for European corn borer (ECB) resistance traits and mycotoxin concentration. The mycotoxin concentration was averaged across insect treatments because treatment differences were not significant

		ECB resistance traits[1]					Mycotoxin concentration[2] (μg/kg)					
Cultivar	Characterization	DRS (1–9)	LPP (n)	LPE (n)	PDP (%)	PDE (%)	DON	15-A-DON	3-A-DON	NIV	FUS-X	FUM
Transgenic												
'Pactol CB *Bt*'	Event 176	1.61	0.04	0	5	2	533.9	164.4	nd[3]	67.6	41.7	45.5
'Valmont *Bt*'	Event 176	1.50	0.03	0	10	2	948.4	277.0	8.1	79.1	30.9	nd
'Novelis *Bt*'	Mont810	1.58	0.01	0	7	1	606.7	126.8	16.3	76.6	18.3	623.5
'Transal *Bt*'	Mont810	1.66	0.04	0	6	0	777.9	422.0	6.7	90.8	39.9	224.0
Mean		1.59a[4]	0.03a	0a	7a	1a	716.7a	247.5bc	7.7a	78.5a	32.7	223.3
Isogenic												
'Pactol'	non-*Bt*	2.74	0.41	0.02	68	11	781.0	178.5	6.1	82.1	76.7	26.5
'Prelude'	non-*Bt*	2.69	0.26	0.01	59	4	888.7	271.2	17.3	82.5	84.8	95.0
'Nobilis'	non-*Bt*	2.81	0.73	0.11	77	38	528.3	140.2	19.8	52.0	45.6	306.0
'Transal'	non-*Bt*	3.06	0.41	0.04	61	14	1271.9	458.1	104.2	126.1	68.7	216.5
Mean		2.83b	0.45b	0.05b	66b	17b	867.5b	262.0ab	36.8b	85.6b	68.9	161.0
Commercial												
'Symphony'	non-*Bt*	3.34	0.42	0.12	80	45	1717.1	382.4	31.02	47.8	107.9	235.5
'Attribut'	non-*Bt*	2.27	0.47	0.02	64	21	425.9	151.1	27.80	63.7	24.8	564.0
'Clarica'	non-*Bt*	2.28	0.24	0.02	58	18	376.6	52.7	5.40	103.8	31.2	65.5
P009 × L007	non-*Bt*	2.68	0.23	0.01	53	11	686.8	172.5	15.85	62.7	25.2	135.0
P030 × L007	non-*Bt*	2.94	0.31	0.05	55	10	759.1	194.3	nd	46.3	35.0	119.5
P033 × L007	non-*Bt*	3.01	0.23	0.03	49	11	1517.8	365.0	50.05	94.9	58.2	444.5
D67 × L007	non-*Bt*	2.77	0.38	0.05	60	14	538.9	178.4	5.45	95.1	54.9	67.5
Mean		2.61b	0.38b	0.06b	66b	26c	764.6a	191.1c	17.4ab	77.6b	54.7	233.1
Pos. samp. (%)[5]		–	–	–	–	–	82.0	59.3	9.0	26.7	33.0	19.7
Total mean		2.45	0.28	0.03	48	14	823.9	235.6	20.9	49.6	78.1	211.2
Genetic ratio (GR)		0.80	0.75	0.66	0.94	0.86	0.65	0.63	0.16	0.44	0.41	0.52

[1] DRS = damage rating of stalks; LPP = number of larvae per plant; PDP = percentage of damaged plants; LPE = number of larvae per ear; PDE = percentage of damaged ears.
[2] DON = deoxynivalenol; 3-A-DON = 3-acetyldeoxynivalenol; 15-A-DON = 15-*O*-acetyl-4-deoxynivalenol; NIV = nivalenol; FUS-X = fusarenon-X; FUM = fumonisin.
[3] nd = not detectable in all five locations. For further calculations, the mean was adjusted to 0. Limits of detection: 30 μg/kg (DON, NIV), 73 μg/kg (FUS-X), 96 μg/kg (3-A-DON), 100 μg/kg (FUM) and 109 μg/kg (15-A-DON).
[4] Means with different letters in columns are significantly different at P = 0.05.
[5] Pos. samp. (%) = percentage of entries showing samples above the detection limit.

Table 3: Means for insecticide protected and European corn borer (ECB)-infested plots evaluated in four locations for experiment 1 and five locations for experiment 2

	Mycotoxin concentration[1] (μg/kg)											
	DON		15-A-DON		3-A-DON		NIV		FUS-X		FUM	
Location	Protected	Infested	Protected	Infested	Protected	Infested	Protected	Infested	Protected	Infested	Protected	Infested
Experiment 1												
Auggen	–	40.9[a2]	–	11.2[a]	–	27.4	–	2.9	–	–	–	–
Eckartsweier	–	153.2[b]	–	40.3[bc]	–	35.3	–	30.3	–	–	–	–
Kandel	–	142.7[c]	–	26.8[bc]	–	35.0	–	4.7	–	–	–	–
Trebur	–	132.1[d]	–	17.4[ac]	–	22.9	–	13.2	–	–	–	–
Mean	–	117.2	–	23.9	–	30.2	–	12.8	–	–	–	–
Experiment 2												
Eckartsweier	2062.6	1292.9	631.8	379.1	14.0	50.1	77.0	91.2	21.1	28.6	54.7	31.3
Kandel	976.4	1040.8	305.1$_a$	153.1$_b$	54.6	29.6	28.8	81.7	14.6	24.4	518.0$_a$	1299.7$_b$
Trebur	1104.9	1000.9	244.0	215.8	19.8	5.5	52.2	68.5	13.0	12.4	22.3	80.7
Freising	196.1	65.1	176.8	8.2	3.3	4.7	41.0	24.6	368.1	294.9	nd[3]	12.7
Ingolstadt	176.8	322.8	99.7$_a$	142.4$_b$	5.4	22.3	5.5	25.2	nd	3.2	nd	93.0
Mean	903.4	744.5	291.5	179.7	19.4	22.4	40.9	58.2	83.4	72.7	119.0a	303.5b

[1] DON = deoxynivalenol; 3-A-DON = 3-acetyldeoxynivalenol; 15-A-DON = 15-*O*-acetyl-4-deoxynivalenol; NIV = nivalenol; FUS-X = fusarenon-X; FUM = fumonisin.
[2] Means with different superscript letters are significantly ($P < 0.05$) different in columns and means with different subscript letters are significantly ($P < 0.05$) different in adjacent rows.
[3] nd = no detectable in both replications. For further calculations, the mean was adjusted to 0. Limits of detection: 30 μg/kg (DON, NIV), 73 μg/kg (FUS-X), 96 μg/kg (3-A-DON), 100 μg/kg (FUM) and 109 μg/kg (15-A-DON).

was higher in experiment 1 than in experiment 2. This indicates that ECB larval feeding is of secondary importance for initiating a successful *Fusarium* inoculation, but environmental factors and plant morphology may play a more important role in favouring alternative pathways of inoculum dispersal and plant colonization.

The spectrum of mycotoxins produced in maize kernels depends on the *Fusarium* spp. and strains present in the field. In comparison with the protected plots, ECB infestation resulted in a doubling of the FUM concentration in Kandel, whereas all other mycotoxin concentrations at this location were comparable to the other experimental sites of the Rhine valley. Lew et al. (1991) compared the occurrence and amount of *Fusarium* spp. grown on ears damaged by ECB larval feeding with the composition of *Fusarium* populations developed on undamaged ears. Their results showed that ECB larval feeding favoured specific *Fusarium* spp., whereas other *Fusarium* species were suppressed or appeared to be unaffected. FUM is produced solely by *F. verticilloides*, which was found to be favoured by ECB larval feeding (Lew et al. 1991, Munkvold et al. 1999). Because the concentration of FUM under protection was substantially higher at Kandel than at all other locations, this suggests a high frequency of *F. verticilloides* within the *Fusarium* population at this site.

In conclusion, under central European conditions, ECB larval feeding may have an effect on the composition of the *Fusarium* population that colonize the maize ear, but since most of the *Fusarium* spp. produce mycotoxins, ECB resistance will not necessarily reduce the total mycotoxin concentration. However, it can affect the relative contribution of the various mycotoxins to the total toxin concentration. Thus, mycotoxin concentrations can be reduced by *Bt* hybrids only, if the *Fusarium* population is dominated by *Fusarium* species that are promoted by ECB damage.

Association between ECB resistance and mycotoxin concentration

Event 176 vs. *Mon810*

In experiment 1, a trend was detected towards higher DON concentrations in event 176 hybrids than in Mon810 hybrids. However, the isogenic counterparts of the Mon810 hybrids also showed lower DON concentrations than the isogenic counterparts of the event 176 hybrids. Therefore, a high level of ECB resistance will reduce the DON concentration only slightly. This indicates that the genotype-specific level of resistance against *Fusarium* is of greater importance for reducing mycotoxin contamination of maize kernels than a high level of ECB resistance. However, as the results of experiment 2 showed, if *Fusarium* attacks are severe and the specific year and environmental conditions favour *Fusarium* growth, neither a high level of ECB resistance nor the observed levels of *Fusarium* resistance prevent the production of high mycotoxin concentrations.

Bt vs. *non-Bt*

The mean DON concentration was significantly ($P < 0.05$) lower in *Bt* hybrids than in their isogenic counterparts in experiment 2. This result is mainly due to the high DON concentration of 'Transal', the isogenic counterpart of 'Transal *Bt*'. For all other hybrid pairs, the DON concentrations were of similar magnitude. Munkvold et al. (1999) reported that under Corn Belt conditions of the USA, the use of *Bt* maize

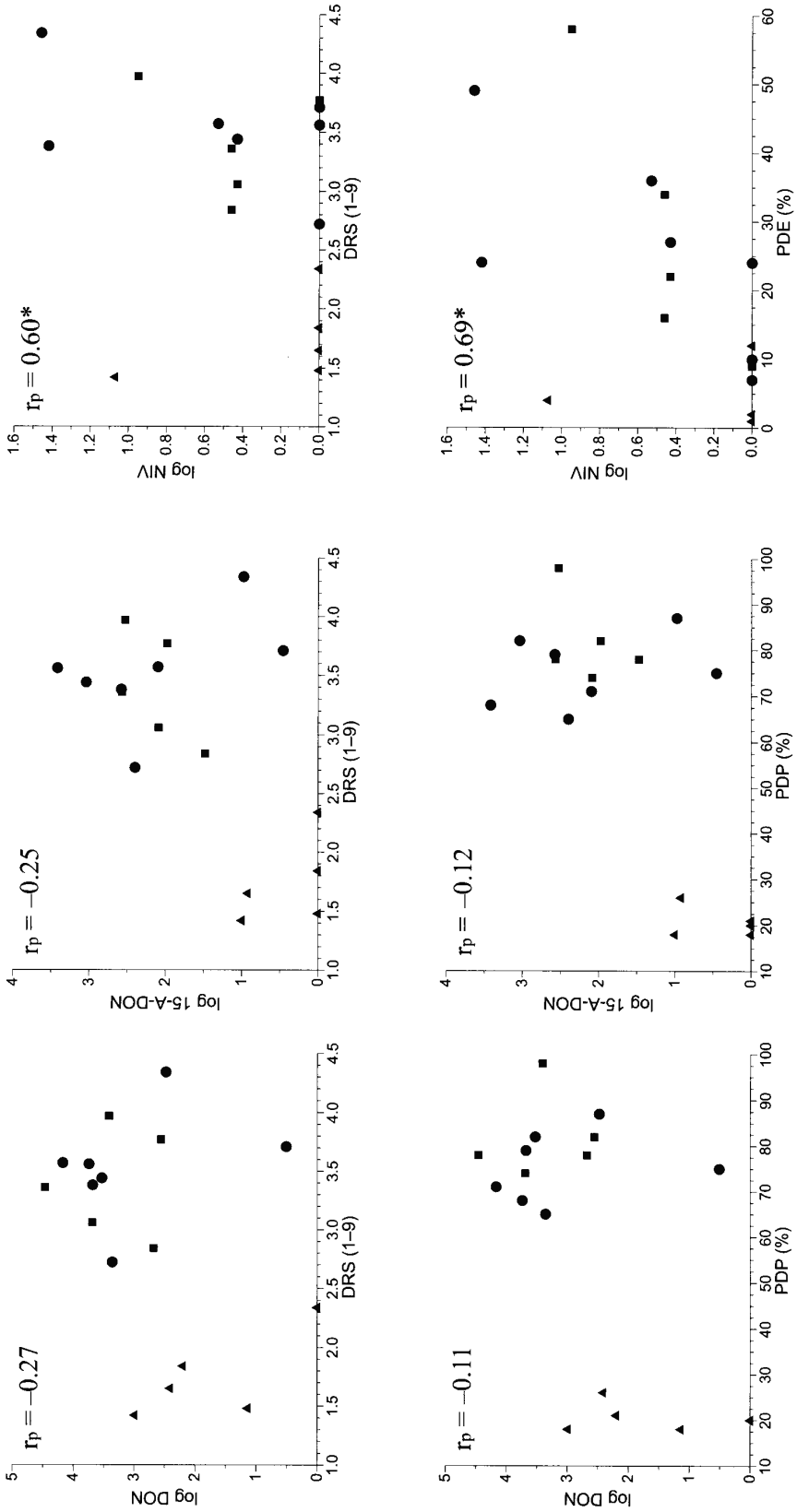

Fig. 1: Plots of stalk damage rating (DRS), percentage damaged plants (PDP), and percentage damaged ears (PDE) with log-transformed values of deoxynivalenol (DON), 15-acetyl deoxynivalenol (15-A-DON), and nivalenol (NIV) concentrations in experiment 1. Plots were based on entry means of individual hybrids across locations. The different hybrid groups are displayed as follows: ▲, *Bt* hybrids; ■, isogenic hybrids; ●, commercial hybrids. The correlation coefficients were calculated only for non-*Bt* hybrids to avoid group effects caused by the highly insect resistant *Bt* hybrids.
* Significant at P = 0.05

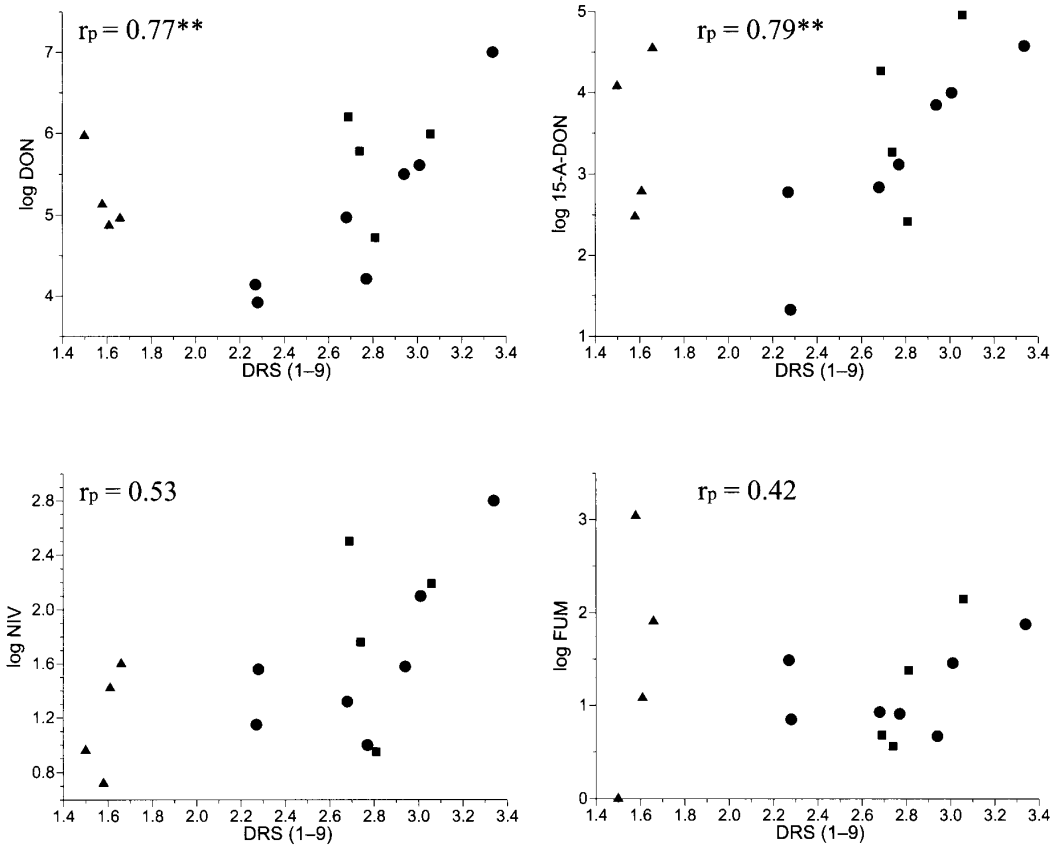

Fig. 2: Plots of stalk damage rating (DRS) with log-transformed values of deoxynivalenol (DON), 15-acetyl deoxynivalenol (15-A-DON), nivalenol (NIV) and fumonisin (FUM) concentrations in experiment 2. Plots were based on entry means of individual hybrids across locations. The different hybrid groups are displayed as follows: ▲, *Bt* hybrids; ■, isogenic hybrids; ●, commercial hybrids. The correlation coefficients were calculated only for non-*Bt* hybrids to avoid group effects caused by the highly insect resistant *Bt* hybrids. ** Significant at P = 0.01

may considerably reduce FUM concentration (Munkvold et al. 1999). In Austria, Lew et al. (1991) showed that the concentration of moniliformin in maize kernels obtained from ears damaged by ECB larval feeding was significantly higher than the concentration obtained from healthy ears. Therefore, it can be expected that *Bt* maize grown under Central European conditions may be less contaminated with moniliformin. However, in the present study, the use of *Bt* maize hybrids did not reduce the amount of DON in the kernels. This was expected, since the DON and NIV producers, *F. graminearum* and *F. culmorum*, are common *Fusarium* species in central Europe and occur at high frequencies on maize ears not damaged by ECB larvae (Lew et al. 1991).

Commercial hybrids

Within the group of commercial hybrids a substantial variation for DON and 15-A-DON concentration was found. Next to hybrids with exceptionally high mycotoxin concentrations, two hybrids with lower mycotoxin concentrations than the best *Bt* hybrid were found. However, the ranking of hybrids common to both experiments with regard to their mycotoxin levels differed across both years. Therefore, further studies are needed to evaluate the level of resistance against *Fusarium* rots within early-maturing European maize germplasm.

Although mycotoxin concentrations were high in experiment 2, only a few *Fusarium* ear rot symptoms were found (data not shown). This is in agreement with reports of symptomless infections by *Fusarium* spp. in maize (Munkvold et al. 1997) and wheat (Mesterházy et al. 1999). These results show that owing to the low association between *Fusarium* disease symptoms and mycotoxin production, a routine evaluation of mycotoxin concentrations is mandatory for selecting maize genotypes with improved resistance against *Fusarium* spp. and reduced mycotoxin concentrations. However, mycotoxin evaluations are currently costly and time-consuming and before breeding programmes for improving resistance against *Fusarium* spp. can be initiated, rapid screening tests allowing a high throughput of samples are urgently required.

In this study, the use of *Bt* hybrids did not substantially reduce the contamination of maize kernels with mycotoxins produced by *Fusarium* spp. This result indicates that resistance against ECB and resistance against *Fusarium* rots are inherited independently. In order to improve each trait, separate breeding programmes are necessary. In 2000, mycotoxin

concentrations varied considerably and about 31% of the entries displayed DON values above recommended thresholds. Therefore, breeding efforts are urgently needed to improve *Fusarium* resistance of maize hybrids rapidly in order to support farmers in meeting future legal regulations for warranting healthy food and feed.

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Concentration of moniliformin produced by *Fusarium* species in grains of transgenic *Bt* maize hybrids compared to their isogenic counterparts and commercial varieties under European corn borer pressure

T. MAGG[1], M. BOHN[2,3], D. KLEIN[1], V. MERDITAJ[1] and A. E. MELCHINGER[1]

[1] Institute of Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, D-70593 Stuttgart, Germany; [2] Department of Crop Sciences, University of Illinois, 1102 South Goodwin Avenue, Urbana, IL 61801, USA; [3] Corresponding author. E-mail: mbohn@uiuc.edu

With 4 tables

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Abstract

The European corn borer (ECB), *Ostrinia nubilalis* Hb., is a major pest of maize in Central Europe and is suspected to promote infection of maize with *Fusarium* species. The objectives of this study were to (1) determine moniliformin (MON) concentration in early maturing European *Bt* maize hybrids, their isogenic counterparts, commercial cultivars and experimental hybrids, (2) evaluate the association between MON concentration and ECB resistance and (3) correlate MON concentration with concentrations of other mycotoxins determined from the same plant materials. The field experiments were performed at five locations in Germany. MON concentration was significantly higher with manual infestation of ECB larvae (296 µg/kg) than under insecticide protection (66 µg/kg). *Bt* hybrids showed significantly lower MON concentrations and higher grain yields under manual ECB infestation than their corresponding isogenic counterparts, as well as commercial and experimental hybrids. All ECB resistance traits and grain yield under ECB infestation were significantly correlated with MON concentration. Correlations between concentrations of MON and other *Fusarium* mycotoxins were not significant. The use of *Bt* maize hybrids or insecticides to control ECB reduces the contamination of maize grains with MON in Central Europe. The presence of resistance genes against *Fusarium* species in the current elite maize germplasm was indicated by ECB susceptible non-*Bt* hybrids with low-MON concentrations.

Key words: *Bacillus thuringiensis* — *Ostrinia nubilalis* — *Zea mays* — transgenic maize

Fusarium species cause diseases of seedlings, roots, stalks and kernels in the maize plant resulting worldwide in serious yield losses. In addition, they produce secondary metabolites causing physiological and toxicological responses in humans, animals and plants. At least 15 *Fusarium* species are capable of producing moniliformin (MON), including *Fusarium avenaceum*, *F. fujikuroi*, *F. moniliforme*, *F. oxysporum*, *F. proliferatum* and *F. subglutinans* (Marasas et al. 1984, 1986, Abbas et al. 1989, Adler et al. 1990, Chelkowski et al. 1990, Lew et al. 1991, Chandran et al. 1993). MON adversely affects human and animal health (IARC 1993), harming especially the muscles of the skeleton and the heart (Engelhardt et al. 1989, Reams et al. 1997). In addition, toxicity of MON and other *Fusarium* mycotoxins, e.g. type B Trichothecenes (TCTC), Zearalenon (ZEN) and Fumonisins (FUM), is cumulative (Morris et al. 1999).

In Europe, contamination of maize grains with mycotoxins produced by *Fusarium* species can be especially observed in regions where wheat–maize rotations or maize monocultures prevail. Warm and humid weather conditions and cultivation of late-maturing maize varieties favour the development of *Fusarium* species (Lew et al. 1991, Vigier et al. 2001). One attempt to control *Fusarium* infections and the production of mycotoxins is the use of fungicides. In some *in vivo* studies, fungicides stopped mycotoxin production whereas other studies reported an increased mycotoxin concentration after fungicide treatments (Hasan 1993). Furthermore, under normal storage conditions mycotoxin concentration is stable and detoxification with physical treatments, such as high temperatures, UV radiation and oxygen are not effective (Patey and Gilbert 1989, Eriksen and Alexander 1998).

The number of mycotoxins and their concentration in maize grains depend on the mixture of *Fusarium* species, the resistance of the host plant against *Fusarium* inoculation, and environmental conditions. However, the development of *Fusarium*-resistant maize genotypes is difficult because (1) inheritance of resistance is polygenic (Snijders 1994, Reid et al. 1999, Perez-Brito et al. 2001), (2) information about the underlying resistance mechanism is scanty and (3) no highly-resistant germplasm sources are available. However, Reid and Hamilton (1997) and Reid (1999) reported the development of several maize lines with improved ear-rot resistance and good agronomic performance derived from an ear-rot-resistant synthetic like.

Fusarium species infect the maize ear by entering the host plant through the silk channel or through wounds caused by insects or birds (Reid et al. 1999, Reid 1999). Therefore, it is assumed that ECB larvae are vectors for *Fusarium* species by causing entry wounds and carrying fungal inoculum from the plant surfaces into the plant. A close association between susceptibility of maize hybrids to ECB feeding damage and the appearance of stalk rot was described by Jarvis et al. (1984). Yet, no information exists on the potential of highly ECB-resistant *Bt* hybrids to reduce the mycotoxin concentration in maize under Central European growing conditions. The objectives of this study were to (1) determine MON concentration in early-maturing European *Bt* maize hybrids, their isogenic counterparts, commercial cultivars from the recommended list and experimental hybrids, (2) evaluate the association between MON concentration and ECB resistance and (3) correlate MON concentration with concentrations of

other *Fusarium* mycotoxins determined from the same plant materials as reported in a companion study (Magg et al. 2002).

Materials and Methods

Plant materials: The maize hybrids used for the evaluation of ECB resistance and mycotoxin concentration were described in detail by Magg et al. (2002). Briefly, the experiment comprised 15 entries: two pairs of hybrids, each pair consisting of a *Bt* cultivar carrying event 176 and its corresponding isogenic counterpart, two pairs of transgenic and isogenic hybrids with event Mon810 (Prelude and Nobilis are the isogenic counterparts of Valmont *Bt* and Novelis *Bt*, respectively), three commercial cultivars, and four experimental hybrids with improved host plant resistance against ECB (Table 2). Isogenic, commercial and experimental hybrids are here referred to as 'non-*Bt* hybrids'. The *Bt* hybrids and their isogenic counterparts were provided by Monsanto Agrar Deutschland GmbH (Düsseldorf, Germany) and Syngenta Seeds GmbH (Bad Salzuflen, Germany).

All *Bt* hybrids examined in this study carried the *CryIA(b)* gene. Transformation event Mon810 utilizes a gene promoter, which results in a season-long expression of the *Bt* toxin in all plant tissues (Archer et al. 2000). In contrast, transformation event 176 contains two promoters regulating *Bt* gene expression in green plant tissues and pollen, respectively (Koziel et al. 1993, Estruch et al. 1997).

Field trials: Field trials were conducted at five locations in 2000 (Eckartsweier, Kandel, Trebur, Freising and Ingolstadt) as described by Magg et al. (2002). The sites represent the main maize-growing regions of southern Germany with high natural occurrence of ECB. The experimental design applied at each site was a split plot with two replications. Whole plots consisted of two ECB treatments, one with manual or natural ECB infestation and the other with insecticide protection. In both experiments, subplots were arranged according to an α-design and comprised the genotypes. Further detailed information on manual ECB infestation is given by Magg et al. (2002).

The following resistance traits were determined in the first row of infested plots: (1) damage rating of stalks using a 1–9 damage rating scale, as described by Hudon and Chiang (1991), (2) number of larvae per plant, (3) number of larvae per ear, (4) percentage of plants displaying ECB-feeding damage and (5) percentage of damaged ears, as described in detail by Magg et al. (2002). Agronomic traits recorded for each ECB infested plot were: (1) grain yield in t/ha adjusted to 15.5% grain moisture and (2) grain dry matter in percentages. Only the two centre rows of each plot were harvested.

Analysis of MON: A random sample of 1 kg grain per plot was taken from each subplot of the infested and the protected whole plot. A subsample of about 100 g was ground and meal was taken for the subsequent analyses of mycotoxin concentrations. The concentration of MON was assessed by employing an immunoassay and high-performance liquid chromatography (HPLC) with fluorescence detection as described by Sharman et al. (1991). The limit of MON detection (LOD) was 36 µg/kg and the limit of MON quantification (LOQ) was 120 µg/kg. The analyses of the mycotoxins deoxynivalenol (DON), 15-*o*-acetyl-4-deoxynivalenol (15-A-DON), 3-acetyldeoxynivalenol (3-A-DON), nivalenol (NIV), fusarenon-X (FUS-X) and fumonisin (FUM) were described in detail by Magg et al. (2002). All mycotoxin analyses were performed by the Institute for Agrobiotechnology at Tulln, Austria.

Sampling experiment: A separate experiment was conducted to determine the effects of sampling and to evaluate the accuracy of the MON quantification. First, the whole set of samples were analysed for MON concentration (sampling date 1, measurement 1). The flour of five randomly-selected non-*Bt* genotypes grown at Kandel, with MON concentrations above the detection limit, was analysed a second time for MON concentration (sampling date 1, measurement 2). Six months later, 300 g of the remaining 700 g of grain samples were re-sampled, ground and subsequently analysed twice for MON concentration (sampling date 2, measurements 1 and 2, respectively).

Statistical analysis: Data of individual plants of each subplot were averaged to obtain a subplot mean for each hybrid and replication. For all traits, an ANOVA for a randomized complete block design combined across locations was performed with these subplot mean values because all resistance traits were evaluated exclusively in the ECB-infested whole plots. In addition, the agronomic traits and the MON concentrations were analysed as a split-plot design across locations to test for differences between ECB-infested and insecticide-protected plots. Because the residual error terms of the mycotoxin data did not follow a normal distribution, the data were log-transformed (Grimm 1960).

In the statistical models, genotypes and ECB treatments were considered as fixed effects, whereas the locations and the genotype × location interactions were considered as random effects. The sum of squares for genotypes were subdivided to test for linear contrasts among the following groups: (1) transgenic vs. isogenic hybrids, (2) transgenic vs. commercial hybrids and (3) isogenic vs. commercial hybrids. Here, the group of commercial hybrids also included the experimental hybrids. For the analyses of the sampling experiment, a model with fixed effects was chosen to test for differences between sampling dates and measurements.

Least significant differences (LSDs) were calculated to test for differences between individual entry mean values. The variance components due to genotypes ($\hat{\phi}_g^2$, for definition of variance as a result of fixed effects, see Scheffé 1959, p. 264) and genotype × location interactions ($\hat{\sigma}_{gl}^2$) were calculated as described by Snedecor and Cochran (1980). The genetic ratios, as a proportion of the genetic variance vs. the total phenotypic variance, were calculated as described by Dhillon et al. (1990). Estimates of ϕ_g^2 and the genetic ratio apply only to the specific group of genotypes investigated here. Phenotypic correlations among traits based on entry mean values were calculated across locations separately for each experiment using established procedures (Snedecor and Cochran 1980). Correlation coefficients were calculated with and without *Bt* hybrids in order to identify stratification effects. No significant stratification effects were found, therefore, the whole set of genotypes, comprising *Bt* and non-*Bt* hybrids was used for calculating correlation coefficients.

All necessary ANOVA computations were performed with the software package PLABSTAT (Utz 1998). Contrasts were computed with the procedure PROC GLM for mixed models as implemented in SAS (SAS Institute 1988).

Results

Moniliformin

The MON concentration significantly ($P < 0.05$) varied between locations and treatments (Table 1). Across treatments, the highest MON concentration was found in Kandel (457.8 µg/kg) and the lowest in Ingolstadt (8.7 µg/kg). Across locations, the mean MON concentration under insecticide protection (66.2 µg/kg) was significantly ($P < 0.01$) lower than under ECB infestation (296.0 µg/kg). Under insecticide protection, 36% of the samples showed a detectable MON concentration, whereas under ECB infestation 84% of the samples were above the detection limit (Table 2). Under ECB infestation, *Bt* hybrids showed significantly ($P < 0.05$) lower MON concentrations than isogenic hybrids and commercial hybrids. No significant differences were detected under protected conditions for all hybrid groups. The genetic ratio estimates for MON concentrations were moderate for both ECB treatments. The estimate of $\hat{\phi}_g$ was significant ($P = 0.05$)

for MON, whereas the estimate of $\hat{\sigma}_{gl}$ was not significant ($P < 0.01$) (Table 3).

Agronomic traits

Grain yield was significantly ($P < 0.05$) higher in ECB-protected plots (data not shown) than in plots under ECB infestation (11.2 t/ha). *Bt* hybrids showed significantly ($P < 0.05$) higher grain yields under infestation than commercial hybrids. The mean grain dry matter was 68.2% and no significant differences between the hybrid groups were detected for this trait. Estimates of the genetic ratio were high for grain yield (0.89) and grain dry matter (0.92) under ECB infestation, respectively.

Table 1: Mean concentration of moniliformin for insecticide-protected and ECB-infested plots averaged across 15 maize hybrids evaluated in five locations

	Moniliformin[1]	
Location	Protected (µg/kg)	Infested (µg/kg)
Eckartsweier	64.8^{a2}_{ac}	297.1^{b}_{a}
Kandel	132.3^{a}_{ab}	457.8^{b}_{be}
Trebur	89.0^{a}_{abc}	161.4^{b}_{cd}
Freising	36.4^{a}_{cd}	178.8^{b}_{ad}
Ingolstadt	8.7^{a}_{de}	384.8^{b}_{ac}
Mean	66.2^{a}	296.0^{b}

[1] Limit of detection: 36 µg/kg; limit of quantification: 106 µg/kg.
[2] Mean values with different superscript letters are significantly ($P < 0.05$) different in rows and mean values with different subscript letters are significantly ($P < 0.05$) different in columns.

Correlations

Among mycotoxin concentrations, associations were not significant except for the significantly ($P < 0.05$) positive associations between NIV and DON, 15-A-DON and MON, as well as between DON and 15-A-DON ($0.57 \leq r_p \leq 0.76$) (Table 4). From all mycotoxins only MON concentrations were significant ($P < 0.01$) positively associated with all ECB resistance traits ($0.74 \leq r_p \leq 0.84$) and negatively with grain yield under infestation ($r_p = -0.75$). All ECB resistance traits showed significant ($P < 0.01$) correlations ($0.62 \leq r_p \leq 0.91$) amongst each other and significant ($P < 0.05$) negative correlations with grain yield under infestation ($-0.70 \leq r_p \leq -0.59$).

Sampling experiment

Significant ($P < 0.05$) differences were found between mean MON concentrations of sampling date 1 (859 µg/kg) and sampling date 2 (395 µg/kg) (data not shown). The differences between repeated measurements within each sampling date were not significant.

Table 2: Mean values for moniliformin (MON) concentration and agronomic traits for 15 maize hybrids evaluated across five locations

		Moniliformin		Agronomic traits[1]	
Hybrid	Characterization	Protected (µg/kg)	Infested (µg/kg)	GYI (t/ha)	GDI (%)
Transgenic					
Pactol CB *Bt*	Event 176	41.0	127.6	11.7	69.0
Valmont *Bt*	Event 176	49.8	134.0	12.3	68.9
Novelis *Bt*	Mon810	77.6	218.8	11.7	65.8
Transal *Bt*	Mon810	27.9	133.5	12.6	65.9
Mean		49.1 a[4]	153.5 a	12.1 a	67.4 a
Isogenic					
Pactol	non-*Bt*	35.8	345.9	11.1	68.9
Prelude	non-*Bt*	102.8	285.3	11.4	69.2
Nobilis	non-*Bt*	112.2	539.7	10.7	66.8
Transal	non-*Bt*	146.5	175.8	11.9	65.9
Mean		99.3 a	336.7 b	11.3 ab	67.7 a
Commercial and experimental[2]					
Symphony	non-*Bt*	60.4	808.1	10.0	70.6
Attribut	non-*Bt*	163.9	390.2	10.6	66.5
Clarica	non-*Bt*	4.1	216.7	12.3	68.2
P009 × L007	non-*Bt*	nd[5]	226.9	10.4	68.9
P030 × L007	non-*Bt*	17.9	168.8	10.1	70.0
P033 × L007	non-*Bt*	96.7	389.5	10.8	65.8
D67 × L007	non-*Bt*	57.1	279.0	10.7	67.2
Mean		42.9 a	266.1 b	10.7 b	68.2 a
Pos. samp. (%)[3]		36	84	–	–
Total mean		66.2	296.0	11.2	67.8
Least significant difference 5%		155.8		0.9	1.3
Genetic ratio (GR)		0.60	0.57	0.89	0.92

[1] GYI = Grain yield of infested plots; GDI = Grain dry matter of infested plots.
[2] Symphony, Attribut and Clarica are commercial hybrids; P009 × L007, P030 × L007, P033 × L007 and D67 × L007 are experimental hybrids.
[3] Pos. samp. (%) = Percentage of entries showing samples above the detection limit.
[4] Mean values with different letters in columns are significantly different at $P = 0.05$.
[5] nd = not detectable in all five locations. For further calculations, the mean concentration was set to 0. Limit of MON detection: 36 µg/kg, limit of MON quantification: 106 µg/kg.

Table 3: Estimates of squared genotypic effects ($\hat{\phi}_g^2$), genotype × location interactions variance components ($\hat{\sigma}_{gl}^2$), as well as genetic ratios (GR) of *log*-transformed concentrations of seven mycotoxins for 15 maize hybrids evaluated across five locations

Mycotoxin[1]	$\hat{\phi}_g^2$	$\hat{\sigma}_{gl}^2$	GR
MON	0.498**	0.284	0.68
FUM	0.299*	0.729**	0.52
DON	0.469**	0.757**	0.65
15-A-DON	0.633**	0.786**	0.63
3-A-DON	0.022	0.098	0.16
NIV	0.162†	0.092	0.44
FUS-X	0.053†	0.036	0.41

†, *, ** Significant at P = 0.1, P = 0.05 and P = 0.01, respectively.
[1] MON, moniliformin; FUM, fumonisin; DON, deoxynivalenol; 3-A-DON, 3-acetyldeoxynivalenol; 15-A-DON, 15-*o*-acetyl-4-deoxynivalenol; NIV, nivalenol; FUS-X, fusarenon-X.

Discussion

Fusarium species cause severe root, stalk and ear-rots by infecting plants through silk channels or tissue wounds (Jarvis et al. 1984, Lew et al. 1991, Lew 1993, Nankam and Pataky 1996, Reid et al. 1996, 1999, Munkvold et al. 1997). Considering the importance of tissue wounds for *Fusarium* species successfully to inoculate maize it is assumed that ECB-resistant maize genotypes are less prone to *Fusarium* infection and, as a consequence, contain smaller quantities of *Fusarium* mycotoxins.

MON concentration and ECB resistance

In this study, the concentrations of MON in early-maturing European *Bt* and non-*Bt* hybrids were assessed under insecticide protection and manual infestation with ECB larvae. The results confirmed, based on indirect evidence, the proposed interlocking of the life cycles of ECB and *Fusarium* species: (1) mean MON concentrations were significantly (P < 0.01) higher in ECB-infested plots than in insecticide-protected plots across all locations, (2) concentration of MON showed a moderate to high association with ECB resistance traits, e.g. *Bt* hybrids displayed the highest resistance to ECB larvae feeding and were also less contaminated with MON than non-*Bt* hybrids. A similar relationship between ECB and *Fusarium* species was also reported by Munkvold et al. (1997, 1999), who found higher levels of ear-rot produced by *F. verticillioides* and *F. proliferatum* and FUM concentrations in kernels of non-*Bt* hybrids compared with their *Bt* counterparts in the USA Corn Belt. The same association between ECB feeding and the concentration of FUM was also revealed in a companion study (Magg et al. 2002, Table 2).

In contrast, the results of the present study were less clear regarding the associations between ECB larvae feeding damage and concentration of TCTCs. The mean concentrations of individual TCTCs were not significantly different between insect treatments (Magg et al. 2002). These findings are in agreement with the observation that ECB larvae damage in kernels selectively favours the occurrence of *F. subglutinans* and *F. verticillioides* (Lew et al. 1991); both *Fusarium* species are known to produce MON and FUM (Koehler 1959, Shurtleff 1984).

The results showed that a strong resistance to ECB larvae feeding indirectly reduces MON and FUM concentrations. Although, cultivation of maize hybrids carrying the *Bt* gene, or hybrids with improved non-transgenic host plant resistance to ECB, effectively reduce MON concentrations, numerous other pathways for infection with *Fusarium* species do exist. These pathways are independent of insect damage and, therefore, unaffected by the presence or absence of the *Bt* gene. Interestingly, highly significant differences between ECB-susceptible non-*Bt* hybrids were identified too for MON, FUM, DON and 15-A-DON concentrations. The average MON concentration of the non-*Bt* hybrids Transal and P030 × L007 were not significantly higher than the mean MON concentration of *Bt* hybrids under ECB infestation. Therefore, it can be

Table 4: Phenotypic correlation coefficients between *log*-transformed mycotoxin concentrations, European corn borer (ECB) resistance traits and agronomic traits under ECB infestation for 15 maize hybrids evaluated across five locations

	Mycotoxin concentration[1]							Resistance traits[2]					Agronomic traits[3]	
	MON	FUM	DON	15-A-DON	3-A-DON	NIV	FUS-X	DRS	LPP	LPE	PDP	PDE	GYI	GDI
MON		0.17	0.38	0.34	0.31	0.57*	0.10	0.77**	0.84**	0.74**	0.83**	0.80**	−0.75**	0.21
FUM			0.02	−0.09	0.50	−0.11	−0.13	0.20	0.11	0.31	0.09	0.33	−0.07	−0.30
DON				0.58*	−0.30	0.76**	−0.04	0.51	0.09	0.26	0.30	0.18	−0.34	0.58*
15-A-DON					−0.23	0.71**	0.26	0.38	0.13	0.24	0.14	0.09	−0.21	0.23
3-A-DON						−0.16	0.09	−0.04	0.40	0.13	0.23	0.41	−0.01	−0.43
NIV							0.18	0.58*	0.30	0.30	0.50	0.33	−0.30	0.47
FUS-X								0.05	0.03	−0.17	0.00	−0.12	0.13	−0.58*
DRS									0.73**	0.67**	0.88**	0.62*	−0.70**	0.25
LPP										0.75**	0.89**	0.79**	−0.59*	0.01
LPE											0.67**	0.91**	−0.60*	0.23
PDP												0.76**	−0.65**	0.24
PDE													−0.59*	0.22
GYI														−0.40
GDI														

*, ** Phenotypic correlations significant at P = 0.05 and P = 0.01, respectively.
[1] *Log*-transformed values of MON, moniliformin; FUM, fumonisin; DON, deoxynivalenol; 3-A-DON, 3-acetyldeoxynivalenol; 15-A-DON, 15-*o*-acetyl-4-deoxynivalenol; NIV, nivalenol and FUS-X, fusarenon-X.
[2] DRS, Damage rating of stalks; LPP, Number of larvae per plant; LPE, Number of larvae per ear; PDP, Percentage of damaged plants; PDE, Percentage of damaged ears.
[3] GYI, Grain yield of infested plots; GDI, Grain dry matter of infested plots.

concluded that moderate resistance against *Fusarium* species can be found in the current elite breeding material.

In this study, no significant associations were found between the concentrations of MON and the other mycotoxins analysed. This is in contrast to reports that showed MON-producing *Fusarium* species to suppress *F. graminearum* and *F. culmorum* dispersion in host plants, which are specialized producers of TCTCs and ZEN (Lew et al. 1991, Reid et al. 1999). The current results are also in disagreement with studies reporting a significant accumulation of DON or ZEN (Valenta et al. 2002) after ECB larvae feeding. These discrepancies may be partly explained by the fact that in the latter study ears were first visually grouped into those displaying ECB larvae feeding damage and those with no visible ECB symptoms and subsequently the subsamples were analysed separately.

MON concentration and grain yield

A significantly ($P < 0.01$) negative correlation was identified between MON concentration and grain yield under ECB infestation. This correlation was expected because grain yield under ECB infestation and MON concentrations both depend on the ECB resistance of the maize hybrids examined. However, the experimental set-up did not allow separation of yield losses caused by ECB larvae feeding from those caused by *Fusarium* infestations.

Improvements for resistance-breeding against *Fusarium* species

For a detailed interpretation of the mycotoxin concentrations detected it is necessary to quantify the fungal tissue present in the host plant. Often a visual damage rating fails to identify *Fusarium* diseases, because some *Fusarium* strains may infect plants without causing visible symptoms (Smith and White 1988, Munkvold et al. 1997, Magg et al. 2002). In the present experiment, symptoms of *Fusarium* diseases were not detectable in both years, although high mycotoxin concentrations were detected. Therefore, in order to avoid the selection of false positives, the measuring of mycotoxin concentrations is an essential tool for improving breeding strategies against *Fusarium* species in maize. However, the production of exact data for the effective identification of resistant genotypes is expensive and time-consuming. In general, it is necessary to perform field trials at multiple locations and to employ HPLC or gas chromatography methods to determine mycotoxin concentrations. If the breeding programme focuses on the resistance against MON-producing *Fusarium* species, the number of test environments can be reduced because genotype × environment interactions were not significant for MON concentrations. As a result, more resources will be available for mycotoxin evaluations. However, other important mycotoxins showed highly significant genotype × environment interactions (Table 3). Therefore, a cheap and reliable method for mycotoxin quantification with a high-throughput method is mandatory to allow for the intense screening of a large number of genotypes. This study has shown that the HPLC method applied produced reliable mycotoxin measures. Correlations between repeated measurements of MON concentrations within and between sampling dates were high in the trial, indicating high repeatability. However, Vincelli and Parker (1995) reported an adverse impact of sampling effects on the accuracy of measured mycotoxin concentrations.

The results reported here demonstrate that ECB-resistant maize hybrids are less contaminated with MON, whereas the use of highly ECB-resistant *Bt* maize hybrids has only a slight effect on the concentration of TCTCs in maize grains (Magg et al. 2002). Therefore, one may conclude that *Bt* hybrids do not offer a general solution to the problem of mycotoxin contamination of maize grains under Central European growing conditions. Breeding programmes are needed to directly improve the resistance of maize against *Fusarium* diseases. In order to design effective breeding programmes, new tools for the reliable, cost-efficient, high-throughput determination of mycotoxins must be developed.

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GENERAL DISCUSSION

ECB causes extensive monetary losses in maize production worldwide not only due to yield reduction as a result of larvae feeding, dropped ears, or lodged plants but also due to costs associated with the application of insecticides or biological measures to prevent ECB damage. In addition, stalk and ear rot diseases are often associated with ECB damage and further reduce standability of plants, increase yield losses, and degrade quality of maize grains. Sagers et al. (1997) estimated that in growing seasons with high ECB population densities overall damage could surpass one billion U.S. dollars worldwide. Therefore, effective, ecologically sound, and affordable tools to control ECB must be developed.

Natural host plant resistance against ECB

Only one generation of ECB occurs per growing season in Central Europe. The damage caused by this generation is comparable to that of the second generation ECB larvae in the U.S. Corn Belt. Natural host plant resistance against the second ECB generation is based on three mechanisms: antibiosis, tolerance, and non-preference (Painter 1968, Panda and Khush 1995). The primary resistance mechanism improved by breeding is antibiosis, because it is easy to measure and adversely effects the ECB population. Besides the number of larvae per plant, the evaluation of tunnel length in elite lines or sheath collar feeding are the other most widely used traits for evaluating antibiosis (Guthrie et al. 1978). In addition to antibiosis, tolerance contributes to ECB resistance in conventional breeding material (Guthrie and Barry 1989, Melchinger et al.1998). The level of tolerance can be evaluated by linear regression of grain yield reduction on damage rating of stalks as proposed by Ortega et al. (1980). Non-preference is of secondary importance for practical breeding purposes, because ECB females lay eggs on non-preferred plants, if preferred ones are not available.

Inheritance of ECB resistance

In the U.S. Corn Belt, manual infestation with ECB allowed the identification of a large number of maize germplasm with first generation ECB resistance but only few genotypes with second generation ECB resistance (Klenke et al. 1986, Barry et al. 1991). However, some cultivars, like lines B52 and DE811, show an increased level of ECB resistance as well as reduced yield losses under manual ECB infestation (Guthrie and Russell 1989, Guthrie and Barry, 1989).

In order to develop appropriate breeding schemes to improve ECB resistance in early maturing European maize germplasm, detailed information about the inheritance of the ECB resistance is necessary. Recent studies verified the quantitative inheritance of ECB resistance in early maturing European germplasm (Bohn et al. 2000). Based on generation mean analyses and diallel studies, it was concluded that mainly additive gene action and to a smaller extent also dominance and epistasis were involved in the resistance of maize against ECB larvae feeding damage (Kreps et al. 1998, Bohn et al. 2000). Based on this information, it seemed most promising to initiate recurrent selection programs. Penny et al. (1967) improved resistance against the first ECB generation significantly within three cycles of recurrent selection. However, they presented no information about the agronomic performance of the lines with improved ECB resistance developed from this recurrent selection program. Furthermore, separate breeding programs for improving ECB resistance simultaneously in the flint- and in the dent pool are very laborious and time consuming.

Prerequisites for breeding against ECB

The most important breeding objectives in a commercial maize breeding program are to increase yield, standability, and to improve early maturity to adapt the maize gene pool to the cool climatic conditions of Central Europe. In order to successfully integrate ECB resistance as an additional selection criterion into these breeding programs, the following prerequisites must be met: (1) The level of ECB resistance should be phenotypically easy to determine. This ensures integration into existing breeding programs with little additional work and financial input. Whereas damage ratings of stalks are easy to determine and can be measured with high

accuracy, the evaluation of the tunnel length is labor intensive because its measurement requires longitudinal stalk slicing. (2) Furthermore, the breeding material should possess a high level of genetic variation for the resistance to ECB, and (3) the resistance and agronomic target traits should not be correlated with each other or the association between the traits should point at least into the direction desired by the breeder.

To develop a hybrid with improved resistance against ECB, both parents should possess a high level of resistance, because additive gene action is the predominant mode of inheritance for antibiosis. Due to lacking information about the level of ECB resistance in early maturing European elite maize germplasm an extensive screening program was initiated at the University of Hohenheim (Kreps et al. 1998, Melchinger et al. 1998). Significant genetic variation for agronomic traits as well as for ECB resistance was identified in a set of 115 early maturing European maize lines making further breeding efforts promising (Schulz et al. 1997). Lines displaying resistance associated with low yield reduction under high ECB pressure were identified. In the Hohenheim breeding program, these lines were selected as potential parents for the development of breeding populations to combine high ECB resistance with good agronomic performance as proposed by Melchinger et al. (1998).

The Hohenheim maize breeding program for improving ECB resistance

We evaluated the overall level of ECB resistance of each genotype by determining grain yield differences between manually infested and insecticide protected plots. The grain yield differences are a function of antibiosis, tolerance, and non-preference. In order to separate the effects of antibiosis from the effects attributable to the other resistance components, damage ratings of stalks and tunnel lengths were determined. The Hohenheim breeding program focused on the damage rating of stalks as the primary resistance trait for following reasons. Especially for damage rating of stalks used as the primary selection criteria, heritabilities were high and ECB ratings were highly significantly correlated with grain yield reduction (Kreps et al. 1998). Furthermore, the number of test locations could be reduced, because estimates of genotype×environment interaction variances were mainly non-significant. In addition, damage ratings displayed a closer correlation between line *per se* and TC performance than all other

resistance traits (Kreps et al., 1998). Beyond these reasons, damage rating of stalks could be evaluated with a fraction of the labor input and costs necessary to measure tunnel length or to determine grain yield reduction.

Response to selection

In order to avoid undesired correlated selection responses between agronomic traits and ECB resistance, a two step selection procedure was applied. In the first step, a selection index combining yield and maturity was used (Utz et al. 1978). In the second step, genotypes displaying a high level of ECB resistance were chosen from the previously selected fraction of the population. The breeders challenge resulted in combining important traits, such as early maturity, grain yield, and ECB damage, which all were negatively correlated with each other (Schulz et al. 1997, Kreps et al. 1998, Melchinger et al. 1998, Magg et al. 2001). However, the retrospect evaluation of the Hohenheim breeding program revealed only a minor improvement towards increased levels of ECB resistance in European early maturing Dent germplasm. In the course of our breeding efforts, only line P030 was selected displaying earlier maturity and better ECB resistance than both parental lines. This result can be explained by the independent culling levels employed for the used selection index and the ECB resistance. In conclusion, the population fraction selected based on its index performance did not contain the lines with the highest ECB resistance. In order to increase the probability to identify new lines that combine high index values with improved ECB resistance, repulsion phase linkage between genes involved in the inheritance of the above traits must be broken by allowing for intensive recombination. Furthermore, no flint lines with both improved ECB resistance and better agronomic performance were selected. This could be explained by a relative poor genetic variation for ECB resistance present in the screened flint lines combined with an insufficient general combining ability for the desired agronomic traits.

In order to substantiate the above findings, we estimated the genetic similarity between newly developed lines and their parents using a set of 100 SSR markers equally distributed across the genome and a set of SSRs known to be associated with putative insect resistance gene clusters (Bohn et al. 2000, Papst et al. 2001). Accounting for sampling effects, we found

significant differences between genetic similarity estimates determined with both SSR sets. A comparison of resistance QTL cluster haplotypes based on SSR markers revealed that early maturing parental lines D06 and D67 contributed the majority of the SSR alleles at the chromosomal cluster regions to their offspring. Because of the high weight put on early maturity in our breeding program and the negative association between early maturity and ECB resistance, it can be speculated that the observed differences between genetic similarity values were not caused by selection for improved ECB resistance but by selection for early maturity. If the association between ECB resistance and maturity is not caused by pleiotropy but rather by linkage, it will be difficult by conventional breeding to combine the desired alleles for both traits in a single genotype. However, based on graphical genotypes several S_2 families were detected in a QTL population, which showed a high level of ECB resistance associated with early maturity (Bohn et al. 2000). This demonstrates the potential of molecular markers to identify genotypes with the necessary recombination events between tightly linked QTL for ECB resistance and maturity. In addition, this result underlines the importance of random mating within S_1 populations for improving early maturity and ECB resistance at the same time (Bohn et al. 2000).

Combining ECB resistance with good agronomic performance

Our hypothesis of linkage is supported by findings of Guthrie and Russell (1989) who reported an increased ECB resistance and reduced yield losses under manual ECB infestation in the maize synthetic BS9. This synthetic was improved employing recurrent selection methods. However, the reduced yield losses were not sufficient to compensate for the loss in yield potential. As a result of selection for ECB resistance, ear diameter as well as ear and plant height decreased by the attempt to improve stalk quality, which is an important resistance component against second generation ECB. Comparable to our findings, linkage to alleles of other traits contributing to yield was discussed as a negative response due to direct and indirect selection for ECB resistance during the production of the synthetic. Further reasons may explain the observed decline in yield in their breeding program using recurrent selection methods. Part of the yield reduction could be caused by drift during inbreeding

which was additionally favored by assortative mating and the relative small population size used in this experiment.

The probability of success of a resistance breeding program is a function of the availability of highly resistant germplasm sources. For example, the level of resistance especially in the screened flint lines was too poor to avoid significant yield losses, if respective experimental flint × dent hybrids were challenged with ECB larvae (Kreps, 1998). These findings suggest that it may be unavoidable to extend the search for new ECB resistance sources to maize germplasm not adapted to Central European growing conditions. However, an integration of exotic sources of resistance in adapted elite material requires recurrent selection approaches and is, therefore, time consuming. In further backcrossing or recurrent breeding programs, ECB resistant exotic germplasm such as inbred line B52 or synthetic BS9 from the U.S. Corn Belt could be introduced to increase genetic variability. For example in the U.S. GEM-program (germplasm enhancement of maize; http://www.public.iastate.edu/~usda-gem/gems-0001.htm, confirmed 07.12.2002), researchers reported success from the introgression of conventional ECB resistance, derived from Peruvian maize Pl 503806 (Abel et al. 1995) into a U.S. Corn Belt adapted inbred line B94. The recurrent stiff-stalk synthetic GEMS-0001 was derived from the cross (Pl 503806 × B94) three times backcrossed to B94 and selected for its high yield performance combined with good ECB resistance (Abel et al. 2000).

If breeders could predict the prospects of crosses for inbred line development before producing and testing lines derived from them in field trials, this would greatly increase the efficiency of breeding programs by reduce costly mass-rearing of ECB larvae and concentrating the efforts on the most promising crosses (Utz et al., 2000). Therefore, we recommend increasing the changes of selection by rigorous screening of potential parental lines for high ECB resistance before new source populations are developed. However, the extensive line screening performed by Schulz et al. (1997) and Melchinger et al. (1998) identified only a limited number of lines with ECB resistance. In order to develop new lines combining high ECB resistance with good agronomic performance it seems, therefore, appropriate to initiate recurrent selection programs. These programs will be successful, if lines selected to create the base population will carry different alleles at QTL involved in the

inheritance of the ECB resistance or if they combine different resistance mechanisms. Many studies demonstrated that it was possible to improve ECB resistance markedly in the medium term using recurrent selection (Penny et al. 1967; Chiang and Hudon 1973; Klenke et al. 1986; Anglade et al. 1996). However, limited information is yet available about the agronomic performance of the lines developed through the recurrent selection process and their fate in commercial breeding programs.

In contrast to employ complex breeding schemes to improve natural host plant resistance governed by multiple genes, the introduction of the monogenic *Bt* gene is much easier to facilitate. With the aid of molecular markers it is possible to select lines in a backcross program that carry the *Bt* gene and a maximum amount of the recipient genome (Hospital et al. 1992, Frisch et al. 1999). Due to the dominant mode of gene action displayed by the *Bt* gene, only one of the parental inbreds must carry the *Bt* gene while the other elite line can be selected purely for its agronomic performance.

ECB resistance of *Bt* hybrids

Measures to control ECB damage are only economical, if natural ECB population densities rise above recommended thresholds, determined and disseminated by the local plant protection agencies. Taking both economical and ecological aspects into account, an improved natural host plant resistance would be the optimum way to control ECB populations with damages close to the economic injury levels under Central European conditions. However, well timed and reliable predictions of future levels of ECB population densities and associated yield losses are hard to achieve and not precise. In contrast, if the predicted ECB pressure exceeds the economic threshold, the most effective mean to control ECB larvae feeding is the use of *Bt* hybrids (Koziel et al. 1993, Estruch et al. 1997, Jansens et al. 1997, Pilcher et al. 1997, Sagers et al. 1997, Archer et al. 2000, and Magg et al. 2001). This is in line with findings of Bohn et al. (1998) determined under German growing conditions. In this study, the use of *Bt* hybrids was economical, if ECB exceeded the threshold of 7 to 10% damaged plants.

In our experiments, transgenic maize plants expressing the *CryIA(b)* toxin showed a substantially improved ECB resistance and markedly reduced grain yield reductions in comparison to their isogenic counterparts and commercial hybrids lacking the *Bt* gene. This was also confirmed by our laboratory bioassay (Magg et al. 2001). Studies in the U.S. reported a higher efficiency of transformation event MON810 in controlling ECB damage than event 176 (Archer et al. 2000). They explained this finding by higher toxin levels and improved expression stability of the *CryIA(b)* gene in maize ears of hybrids containing MON810. However, in our studies a significant ($P < 0.05$) higher percentage of damaged ears for hybrids containing event 176 than for hybrids carrying Mon810 was only found in year 2000. Both *Bt* events provided a highly effective ECB control under Central European growing conditions.

Variation of ECB resistance traits and agronomic traits for *Bt* and non-*Bt* hybrids

In general, differences between *Bt* hybrids and their near-isogenic lines were not significant for all agronomic traits under insecticide protection. Under ECB infested conditions all evaluated resistance traits were greatly reduced by the use of *Bt* hybrids. Furthermore, observed yield reductions of *Bt* hybrids were small (-1.2% to 3.5%) compared to yield reduction of non-*Bt* hybrids of up to 30% (Bohn et al. 1998) under ECB infestation. In our experiment, yield losses ranged from 8.6% to 21.8% for non-*Bt* hybrids, indicating that some commercial genotypes were moderately resistant to ECB larvae feeding. This finding was confirmed by significant differences between non-*Bt* hybrids for resistance traits.

Approaches for a *Bt* resistance management system

The major goal of a *Bt* resistance management system is to secure a long lasting effect of resistance genes for a successful control of ECB larvae. A resistance gene management is necessary, because the selection pressure put on ECB larvae by the use of *Bt* maize hybrids is high. The *Bt* gene is expressed at high rates causing a high ECB larvae mortality (Roush and McKenzie 1987). The U.S. Environmental Protection Agency (EPA) (http://www.epa.gov/scipoly/sap/2000/october/brad4_irm.pdf, confirmed 07.12.2002) developed five general strategies for managing resistance to *Bt* expressing crops: (1)

constitutive expression of high levels of single toxins in all plants, (2) constitutive expression of high levels of two or more toxins in all plants, (3) the 'refuge plus high-dose strategy', a spatial or temporal mixture of plants having high levels of constitutive expression of one or more toxins with other plants having no toxin expression, (4) low levels of expression of single toxins interacting with natural enemies, and (5) targeted *Bt* gene expression.

The refuge strategy is based on the critical assumptions that the resistance of ECB against *Bt* is recessive and that random mating occurs between susceptible and resistant ECB individuals. If the *Bt* toxin resistance is recessive, first generation offsprings produced by mating between susceptible and resistant adults are killed after feeding on *Bt* tissue. If mating is random, initially rare homozygous resistant adults emerging from *Bt* tissue are likely to mate with the more abundant homozygous susceptible adults emerging from non-*Bt* plants, producing a first generation offspring progeny that cannot survive on *Bt* plants. Mathematical models and limited data from laboratory and greenhouse studies indicate that resistance can be delayed substantially when these assumptions are valid (Andow et al. 1998, Alstad and Andow 1995, Tabashnik 1994, Onstad and Gould 1998). However, our results of 0.08 to 0.19 surviving larvae per plant and a percentage of damaged stalks ranging from 18 to 31% for plots planted with *Bt* hybrids question some of the assumptions underlying these models (Magg et al 2001). Therefore, an effective resistance management system is essential to prevent resistance of ECB to the *Bt* toxin.

Depending on the climatic conditions, ECB can produce multiple generations per year, which results theoretically in a faster development of resistance of ECB to *Bt* toxin. Development of *Bt* resistance has already been seen in the Indian meal moth (*Plodia interpunctella*) and has been found in ECB laboratory populations (Huang et al. 1997). Nevertheless, development of resistance to *Bt* may be delayed, because untreated areas in a 'refuge plus high-dose strategy' can provide a source of susceptible moths to dilute the buildup of *Bt* resistant genes in the ECB population (Orr and Landis 1997, Andow et al. 1998). Furthermore, under Central European conditions it could be supposed that not every field of maize will be planted to *Bt* maize and geographical mosaics of *Bt* and conventional hybrids or plants will occur. As indicated by our study, it might also be possible that maize ears serve as a refuge for ECB larvae after anthesis in transgenic hybrids expressing low or no

levels of toxins in ears. So we found a clear trend towards higher values of damaged ears for hybrids expressing event 176 than hybrids with event Mon810 in 2000. Therefore, the effects of surviving larvae on the adaptation of ECB to *Bt* maize remains unclear, especially regarding the ear shelter as refuge (Onstad and Gould 1998).

Currently, there are three different *Bt* toxins in use, *i.e., CryIA(b)*, *CryIA(c)*, and *Cry9C*. Therefore, up to three different *Bt* genes could be staked into a single hybrid to mimic a horizontal resistance as proposed by the EPA. However, Tabashnik et al. (1994, 1997) reported 21% heterozygous individuals of a susceptible population of the diamondback moth (*Plutella xylostella*) carrying a gene conferring resistance to several *Bt* toxins. Based on these findings, the appearance of cross resistance could not be excluded in populations of ECB. Perhaps, *Bt* genes could be combined with improved natural host plant resistance to enhance the level of resistance in maize. Therefore, improving quantitative resistance in maize through plant breeding could deliver an economically tool, completing the EPA proposal by building up a horizontal resistance. Furthermore, by improving natural host plant resistance breeders pay tribute to consumer concerns regarding cultivation of genetically engineered plants in the EU.

Association between insect damage and mycotoxins produced by Fusarium spp. in grains

The associations between insects and maize diseases result from several types of host-insect-pathogen interactions. One identified interaction is a vector-like relationship between ECB and *Fusarium* spp.. ECB larvae carry spores of *Fusarium* spp. from the plant surface to the surfaces of damaged kernels or into stalks, where infections are initiated (Jarvis et al. 1984, Lew et al. 1991, Lew 1993, Munkvold et al. 1997, Sobek and Munkvold 1999). Viable spores of *Fusarium* spp. or *Aspergillus* spp. can be found externally, internally, and in the frass of ECB larvae and other insects (Dowd 1998). Especially *Fusarium* spp. (*F. subglutinans*, *F. moniliforme,* and *F. verticillioides* [section *Liseola*]) producing MON and FUM have an affinity to enter through wounds into maize tissue and once entered the plant they also might have antagonistic effects against other *Fusarium* spp. (Koehler 1959, Shurtleff 1984, Lew et al. 1991, Lew 1993, Munkvold et al. 1997, 1999). A second type of interaction between ECB

and Fusarium is the formation of entry wounds for the fungi, caused by larvae feeding on kernels or stalks. Even if the larvae do not directly carry the fungi into the tissue, feeding damage by ECB larvae causes stress that predisposes the plant to weakening parasites such as stalk- or ear rots.

Potential of *Bt* hybrids for reducing mycotoxin concentration

In order to study the association between ECB resistance and important mycotoxins, we evaluated grains of *Bt* hybrids, their isogenic counterparts, and commercial hybrids for their concentration with TCTC-mycotoxins as well as ZEN, FUM, and MON under manual ECB infestation and under ECB protected conditions. In the combined analyses across locations, we found MON concentrations being significantly ($P < 0.01$) higher under ECB infestation than under insecticide protection. This is in agreement with studies performed under Austrian growing conditions, showing significantly higher MON concentrations in ears damaged by ECB larvae feeding than those maize kernels obtained from healthy ears (Lew et al. 1991). In our study, the average MON concentration of the non-*Bt* genotypes was approximately twice as high as the MON concentration in the genotypes carrying the *Bt* gene under manual infestation with ECB larvae. Similar results were reported from Munkvold et al. (1997, 1999) under U.S. Corn Belt growing conditions for FUM concentrations. However, this is in contrast to our results obtained for FUM concentrations in *Bt* hybrids compared to isogenic counterparts under ECB infestation. Nevertheless trends for reduced FUM concentrations under insecticide protected conditions were found. Therefore, an enhancement of FUM concentration under ECB larvae feeding may occur.

For contamination with TCTCs, the observations are contradictory in our study. DON and 15-A-DON concentrations were lower in *Bt* hybrids compared to non-*Bt* hybrids only in the experiments conducted in 1999. Valenta et al. (2002) reported significantly ($P < 0.01$) lower concentration of DON and ZEN in maize ears of *Bt* hybrids compared to non-*Bt* hybrids. However, only ears with visible ECB larvae feeding damage were selected from our experiments in 1999 and were independently analyzed for DON and ZEN concentrations in 2000 (Valenta et al. 2002).

Variation of mycotoxin concentration within non-*Bt* hybrids

Within the group of non-*Bt* hybrids a substantial variation for MON, FUM, DON, and 15-A-DON concentration was found. This indicates that kernel resistance against *Fusarium* spp. exists in the current elite breeding material. Next to hybrids with exceptionally high mycotoxin concentrations, non-*Bt* hybrids with lower mycotoxin concentrations than the best *Bt* hybrid were found for some mycotoxins. It can be hypothesized that the genotype specific level of resistance against *Fusarium* is of greater importance and possesses a greater potential for reducing mycotoxin contamination of maize kernels than a high level of ECB resistance. Therefore, further research should focus on the natural resistance against ear- and kernel rots present in host plants to prevent mycotoxin contamination of grains.

Mycotoxin concentration across locations and genotype × location interactions

In general, average DON and 15-A-DON concentrations were much higher in the experimental sites located in the Rhine valley than in those located in Bavaria in 2000. All other mycotoxin concentrations were close to the detection limit except for FUM and MON in Kandel and FUS-X in Freising. Under Austrian growing conditions, a close association between MON producing *Fusarium* spp. and ECB damaged ears was found (Lew et al. 1991). Following these results, a higher frequency of FUM and MON producing *Fusarium* species could be assumed especially at experimental sites Ingolstadt and Kandel in comparison to strains prevalent at the other locations (Lew et al. 1991, Munkvold et al. 1999).

Genotype×location interactions were high under natural or manual *Fusarium* spp. inoculation in small grains, limiting the success of selection in breeding programs (Miedaner 1997, Miedaner and Reinbrecht 2001, Vigier et al. 2001). In contrast to significant genotype×location interactions for FUM, DON, and 15-A-DON, a non-significant interaction of genotypes with locations was detected for MON and FUM concentrations in our study. Especially the contamination of grains with TCTCs is attributable to the different climatic conditions but might also be due to regional differences between the species and strain

composition of the *Fusarium* populations. Furthermore, the ranking of hybrids common to both experiments with regard to their DON levels differed across both years. Therefore, we concluded, that especially MON producing *Fusarium* species were favored by ECB pressure under Central European growing conditions. An manual inoculation of FUM producing *Fusarium* species seems necessary for further experiments as demonstrated by Munkvold et al. (1999), because of high variances for FUM values, ranging from extremely high FUM concentrations to values below the detection limit.

Prospects for reducing Fusarium spp. mycotoxins in maize

The number of mycotoxins and their concentration found in grain of maize depend on the population of *Fusarium* species, the resistance of the host plant, and the environment. The species *F. graminearum* and *F. culmorum* especially synthesize DON and NIV. Both are common under Central European growing conditions and occur at high frequencies on maize ears not damaged by ECB larvae (Lew et al. 1991). This indicates that in contrast to MON and FUM, the concentration of TCTC mycotoxins is largely independent from ECB larvae feeding. Therefore, differences in mycotoxin contamination between highly resistant *Bt* hybrids carrying event 176 and Mon810 (Munkvold et al. 1999) were of secondary importance under Central European growing conditions. Consequently, mycotoxin concentrations can be reduced by highly ECB resistant maize only, if the Fusarium population is dominated by those species that are promoted by ECB damage, as already demonstrated for the MON and FUM producing Fusarium strains. Furthermore, in our investigations no negative associations were found among the mycotoxins analyzed. Therefore, we could not verify the hypothesis of antagonistic effects between the different Fusarium strains by analyzing relevant mycotoxins. However, as the results in 2000 showed, if Fusarium attacks are severe and environmental conditions favor Fusarium growth, neither a high level of ECB resistance nor the observed low levels of Fusarium resistance prevent the production of high mycotoxin concentrations.

Improvement of Fusarium spp. resistance in maize

Two resistance mechanisms were described in literature against Fusarium infections. The "silk resistance", which acts as a barrier between the fungi and the silks of the host plant and the "kernel resistance", which prevents an expansion of the fungus after penetrating the tissue through kernel wounds created by insects or birds (Nankam and Pataky 1996, Reid et al. 1996, Reid 1999, Reid et al. 1999). Furthermore, it is assumed that the infection of silks is the major mode of entry during epidemics. However, breeding for resistance against *Fusarium* spp. must improve silk and kernel resistance at the same time, because there is no association between resistance to silk and resistance to kernel infection (Reid 1999). In addition, it is supposed that different genes are involved in ECB and *Fusarium* spp. resistance. This was verified through non-significant correlations between ECB resistance traits and mycotoxin concentrations, except for MON under Central European growing conditions (Magg et al. 2003). For this reason, breeding programs are necessary to improve both traits simultaneously.

Hitherto, no complete resistance against *Fusarium* spp. was found in maize. Hence, it is supposed that resistance of maize against *Fusarium* spp. is governed by multiple genes (Snijders 1994, Reid et al. 1999, Perez-Brito et al. 2001). A first success was reported from Canada, where resistance breeding programs against *Fusarium* spp. were initiated. An ear rot resistant synthetic was developed from which several lines with improved Fusarium resistance were selected (Reid and Hamilton 1997, Reid 1999). Several lines highly resistant to antracnose stalk rot (*Colletotrichum graminicola*) (Badu-Apraku et al. 1987) and northern corn leaf blight (*Helminthosporium turcicum*) were developed by backcrossing to the 'Cornell ECB composite'. This indicates a partly common genetic basis of ECB resistance and resistances against antracnose stalk rot or northern corn leaf blight, which were all located in the same genomic regions. Therefore, a comparable resistance mechanism against ECB and the named fungal diseases, derived from exotic germplasm of the 'Cornell ECB composite', should be of special interest for further examinations. Perhaps, resistance against the MON producing *Fusarium* spp. is based on comparable resistance mechanisms as evaluated in a QTL study of Perez-Brito et al (2001). For this reason, it should be verified whether resistance to MON producing *Fusarium* spp. could be enhanced by the use of the 'Cornell ECB composite'.

The association between *Fusarium* spp. disease symptoms and mycotoxin production is low. Therefore, a routine evaluation of mycotoxin concentrations in multiple environments under natural and manual inoculation of the fungi seems to be mandatory for selecting maize genotypes with improved resistance to *Fusarium* spp. (Smith and White 1988, Munkvold et al. 1997). However, current mycotoxin evaluations are costly and time consuming and before breeding programs for improving resistance against *Fusarium* spp. can be initiated, rapid and inexpensive screening tests, allowing a high throughput of samples, are urgently required. Hence, resistance breeding against *Fusarium* spp. represents the most economical and ecological tool for the production of healthy food for human and animal consumption, because chemical methods to control *Fusarium* spp. diseases often fail and some grain samples display mycotoxin concentrations above recommended thresholds (Magg et al. 2002).

Overall conclusion

Does the use of *Bt* hybrids offer a solution to manage ECB damage accounting for more than an annual loss of 1 billion U.S. dollars? On the one hand, *Bt* hybrids presents the most powerful and economical mean to protect maize from ECB damage. Outside the European Union the market and acceptance of genetically modified organisms is growing. Regarding ecological aspects, the use of *Bt* maize should be preferred in contrast to the widespread use of insecticides. Furthermore, *Bt* hybrids may reduce grain contamination with MON and FUM mycotoxins. On the other hand, a resistance management is mandatory, because the monogenic *Bt* resistance could be quickly overcome by the ECB. In case the resistance breaks down, non-transgenic host plant resistance could act as a second barrier for the target pest. Therefore, the design of new, improved breeding strategies against ECB and *Fusarium* spp. damage is of great importance for the future of maize in Central Europe.

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SUMMARY

The European corn borer (ECB, *Ostrinia nubilalis* Hübner) is a major pest of maize (*Zea mays* L.) in Europe and continues to spread to northern maize growing regions. The ECB severely affects commercial maize production by decreasing yield stability. In addition, damaged plants often show an increased susceptibility to secondary infections caused by *Fusarium* spp.. Information about the potential of *Bt* hybrids (event 176, MON810) to reduce yield losses and mycotoxin contamination under Central European growing conditions is still lacking. However, such monogenic resistances with a strong negative effect on the ECB will break down rapidly. Improving the natural host plant resistance of maize could provide an economical and ecological tool for an integrated pest management system.

The overall goal of this study was to evaluate alternative breeding strategies for improving resistance of maize against ECB damage and *Fusarium* spp.. The objectives were to (1) initiate a selection experiment in the early maturing European flint pool and evaluate a breeding program for ECB resistance in the European dent pool, (2) compare the efficiency of host plant resistance *vs. Bt* resistance in maize, (3) determine *Fusarium*-caused mycotoxin contamination of maize genotypes with improved host plant resistance to ECB, and (4) study the association between important agronomic traits, ECB resistance traits, and mycotoxin concentration in early European maize germplasm.

The goal of the Hohenheim ECB breeding program, initiated in 1992, was to select lines with improved *per se* and testcross performance for multiple agronomic traits and ECB resistance. In the standard breeding scheme, line development started from a segregating S1 population. Genotypes were evaluated for their line *per se* ECB resistance in generations S1, S3, and S5. Lines from the S2, S4, and S5 generations were testcrossed and evaluated for their agronomic performance. Selection was based on ECB resistance and TC performance for grain yield and maturity.

In order to compare transgenic *Bt* maize hybrids carrying event 176 or MON810 with their isogenic counterparts and commercial hybrids or experimental hybrids, field trials in

multiple environments were conducted in 1998 to 2000. Furthermore, a laboratory bioassay with neonate ECB larvae was performed to assess mortality and subsequently the level of *Bt* antibiosis present in the used hybrids of 1998.

Resistance traits such as damage rating of stalks, number of damaged plants, and number of larvae per plant were assessed exclusively in manually ECB infested plots. Grain yield, grain dry matter content and plant height were determined in the insecticide protected and the ECB infested main plots. In addition, grain samples from each subplot were drawn at random and analyzed separately for *Fusarium* mycotoxins such as type B trichothecenes (DON, NIV), Zearalenon (ZEN), Fumonisins (FUM), and Moniliformin (MON).

The inbred lines displayed a significant genotypic variance for all ECB resistance traits evaluated. However, in the further course of selection and topcross testing, most dent and flint lines, especially those displaying improved resistance to ECB larvae feeding, were discarded because of their poor agronomic performance. Negative correlations between grain yield, early maturity and the damage rating of stalks were identified. However, three dent lines (P028, P029, P030) with moderate resistance to ECB were developed.

In all experiments, *Bt* hybrids were superior to other hybrids in the control of ECB larvae. Non-*Bt* hybrids displayed a significant genotypic variance for all evaluated resistance traits; grain yield reductions ranged from 8.6 to 21.8% under manual infestation of ECB. All evaluated resistance traits were highly significantly correlated with each other and showed significant negative correlations to grain yield reduction. *Bt* hybrids did not differ from their isogenic counterparts for most agronomic traits.

Highly significant location and genotype × location interactions were identified for all mycotoxins evaluated, except MON. MON concentration doubled under manual infestation of ECB compared to insecticide protected conditions and a similar trend was found for FUM. *Bt* hybrids displayed significantly lower MON concentrations than non-*Bt* hybrids and significantly lower DON concentrations than their isogenic counterparts under ECB infestation. Highly significant correlations between ECB resistance traits and MON were found. However, a significant genotypic variance was observed for DON, 15-A-DON, FUM,

and MON concentrations, suggesting variation for resistance against *Fusarium* spp. in current elite hybrids.

By combining different sources of monogenic *Bt* resistance and quantitatively inherited resistances to ECB, it may be possible to develop hybrids with multiple resistance by pyramiding the underlying genes in one genotype. Therefore, further research is required to identify new sources of ECB resistance and new breeding strategies should be developed. Furthermore, there is indication that an improved resistance against *Fusarium* spp. possesses a greater potential for reducing mycotoxin contamination of maize kernels than a high level of ECB resistance. Since resistance to ECB and resistance to *Fusarium* spp. are inherited fairly independently, simultaneous improvement of both resistances seems to be necessary for improving the stability and quality of future maize hybrids.

ZUSAMMENFASSUNG

Der Maiszünsler (ECB, *Ostrinia nubilalis* Hübner) ist einer der Hauptschädlinge im europäischen Maisanbau (*Zea mays* L.) und breitet sich derzeit weiter in nördlicher gelegene Anbaugebiete aus. ECB-Schäden beeinträchtigen daher zunehmend die Ertragssicherheit in der Maisproduktion. Zusätzlich besitzen die vom ECB befallenen Pflanzen oft eine erhöhte Empfindlichkeit gegenüber den von Fusariumpilzen (*Fusarium* spp.) verursachten Sekundärkinfektionen. Allerdings sind derzeit noch keine Informationen zu *Bt*-Maishybriden (Event 176, MON810) verfügbar, welche eventuell auch unter zentraleuropäischen Wachstumsbedingungen Ertragsverluste und Mykotoxinkontaminationen verringern könnten. Jedoch besteht die Gefahr, dass monogene Resistenzen vom Schadinsekt überwunden werden können. Daher kann die Verbesserung der natürlichen Widerstandskraft der Pflanze ein ökonomisches sowie ein ökologisches Konzept für den integrierten Pflanzenschutz bieten.

Ziel der vorliegenden Studie war die Untersuchung alternativer Züchtungsstrategien zur Verbesserung der Resistenz von Mais gegen ECB und Fusariosen. Hierzu wurden (1) ein Selektionsexperiment im frühreifen Europäischen Flintpool initiiert und ein Peedigreezuchtprogramm zur Verbesserung der ECB-Resistenz im frühreifen Europäischen Dentpool evaluiert; (2) die Wirkung von quantitativen Resistenzen und *Bt*-Resistenz miteinander verglichen; (3) die Mykotoxingehalte in Genotypen mit verbesserter ECB Resistenz ermittelt und (4) der Zusammenhang zwischen wichtigen agronomischen Eigenschaften, der ECB-Resistenz und der Mykotoxinkonzentration im frühreifen europäischen Maismaterial untersucht.

Ziel eines 1992 iniitierten Zuchtprogramms war die Identifizierung von Maislinien mit verbesserter Eigen- und Testkreuzungsleistung für wichtige agronomische Merkmale und ECB-Resistenzeigenschaften. Der Zuchtgang wurde mit einer spaltenden S1 Population begonnen. Die Eigenleistung der Genotypen für die ECB-Resistenz wurde in Generation S1, S3 und S5 ermittelt. Mit den Maislinien der S2, S4 und S5 Generation wurden Testkreuzungen erstellt, um deren agronomische Leistung zu bewerten. Die Selektion basierte auf der ECB-Resistenz und der Testkreuzungsleistung für Kornertrag und Frühreife.

Um die *Bt*-Maishybriden, welche das Event 176 oder MON810 besitzen, mit ihren isogenen Partnerhybriden, sowie Sorten- und Experimentalhybriden zu vergleichen, wurde in verschiedenen Umwelten von 1998 bis 2000 Feldversuche durchgeführt. Darüber hinaus wurde 1998 ein Laborversuch mit neonaten ECB-Larven angelegt, um deren Mortaliät und den vorhandenen Antibiosegrad im Hybridmaterial bestimmen zu können.

Die Resistenzmerkmale Schadensbonitur des Stengels, Anzahl geschädigter Pflanzen und Anzahl der Larven pro Pflanze wurden nur in den manuell mit ECB infestierten Parzellen erhoben. Die agronomischen Merkmale Kornertrag, Korntrockensubstanzgehalt und Wuchshöhe wurden sowohl in den mit Insektizid geschützten als auch in den manuell mit ECB infestierten Großparzellen ermittelt. Zusätzlich wurden Körnerproben aus jeder Parzelle gezogen und separat auf die folgenden Fusarientoxine hin untersucht: Typ B Trichothecene (DON, NIV), Zearalenon (ZEN), Fumonisin (FUM) und Moniliformin (MON).

Alle getesteten Inzuchtlinien zeigten eine signifikante genotypische Variation für die untersuchten Resistenzmerkmale. Jedoch wurden im Verlauf des weiteren Zuchtganges viele Dent- und Flintlinien mit verbesserter ECB-Resistenz aufgrund ihrer unzureichenden agronomischen Leistungen verworfen. Zudem wurden negative Korrelationen zwischen Kornertrag und Frühreife sowie der Schadensbonitur des Stengels gefunden. Jedoch konnten aus dem Zuchtprogramm drei Dentlinien (P028, P029, P030) mit mittlerer ECB-Resistenz entwickelt werden.

In allen Experimenten demonstrierten die *Bt*-Hybriden ihre Überlegenheit in der Kontrolle von ECB. Die nichttransgenen Hybriden zeigten eine signifikante genetische Varianz für die erhobenen Resistenzmerkmale und den Kornertrag, wobei deren Kornertragsreduktion unter manueller ECB-Infestierung zwischen 8,6% und 21,8% schwankte. Alle Resistenzmerkmale waren hoch signifikant miteinander korreliert und zeigten eine signifikante und negative Korrelation zur Kornertragsreduktion. Zudem waren die *Bt*-Hybriden in ihren agronomischen Merkmalen nahezu nicht von den isogenen Partnerhybriden zu unterscheiden.

Hochsignifikante Umwelt- sowie Genotyp × Umwelt-Interaktionen wurden außer bei MON für alle Mykotoxine nachgewiesen. Die MON-Konzentration war in den mit ECB infestierten Parzellen ungefähr zweimal höher als in den insektizidgeschützten Parzellen. Ein ähnlicher Trend wurde ebenfalls für FUM festgestellt. Die *Bt*-Hybriden zeigten signifikant niedrigere MON Konzentrationen als die nichttransgenen Hybriden, sowie signifikant niedrigere DON Konzentrationen als ihre isogenen Partnerhybriden unter ECB-Infestierung. Es wurden hochsignifikante Korrelationen zwischen den ECB-Resistenzmerkmalen und der MON-Konzentration gefunden. Jedoch wurde für DON, 15-A-DON, FUM und MON eine signifikante genetische Variation gefunden, was wiederum zeigt, dass ein unterschiedliches Resistenzniveau gegen *Fusarium* spp. in den Elitehybriden vorhanden ist.

Mit Hilfe einer Pyramidisierung von verschiedenen monogenen *Bt*-Resistenzquellen und der verbesserten quantitativen ECB-Resistenz wäre es prinzipiell möglich, Genotypen mit einer stabilen Resistenz zu schaffen. Um neue Resistenzquellen zu identifizieren und geeignete Zuchtprogramme zu entwickeln, sind allerdings weitergehende Untersuchungen nötig. Desweiteren kann durch eine verbesserte Fusariosenresistenz ein höherer Wirkungsgrad zur Verminderung von Mykotoxinbelastungen im Erntegut erreicht werden als dies derzeit durch ein hohes ECB-Resistenzniveau möglich ist. Da ECB- und Fusariosenresistenz weitgehend unabhängig voneinander vererbt werden, ist eine gleichzeitige züchterische Bearbeitung beider Resistenzen nötig, um die Ertragssicherheit und Qualität künftiger Sorten bei Mais weiter zu verbessern.

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Curriculum Vitae

Name	**Thomas Magg**
Date of Birth	November 23, 1973
Place of Birth	Laupheim, Germany

School education

1980 – 1984	Elementary school, Achstetten, Germany.
1984 – 1993	Completion of the high school degree 'Abitur', Carl-Lämmle-Gymnasium, Laupheim, Germany.

University education

10/1994 – 09/1999	University degree in agronomy 'Dipl.-Ing. sc. agr.', University of Hohenheim, Stuttgart, Germany.
11/1999 – 04/2002	Doctorate Candidate at the Institute for Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany.

Professional Employment

06/1993 – 09/1993	Intern at a pig and cash crop farm, Rot, Germany.
10/1993 – 09/1994	Military service 'Heeresfliegerkommando II', Laupheim, Germany.
04/1996 – 10/1996	Intern at a dairy farm, Mönchhöfe, Germany.
04/1997 – 08/1997	Research Associate, Institute for Plant Production and Grassland Research, University of Hohenheim, Stuttgart, Germany.
11/1999 – 03/2002	Research Assistant, Institute for Plant Breeding, Seed Science and Population Genetics, University of Hohenheim, Stuttgart, Germany.
06/2002 – present	BASF AG, product development, consulting and registration, Limburgerhof, Germany.